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# Report of the Biological Survey of Mutsu Bay.

## 22. Brachiopods of Mutsu Bay.<sup>1)</sup>

By

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Professor of Geology and Palaeontology, Taihoku Imperial University, Taiwan.

(With Plates I II)

(Received Oct. 16th, 1931).

### INTRODUCTORY.

This paper is intended to describe the Brachiopods of Mutsu Bay, collected by Prof. HÓZAWA and other members of the Biological Institute of the Tōhoku Imperial University. The valuable material was sent to me more than half a year ago. Owing, however, to the unsatisfactory library, I could not but wait some time for some publications to arrive. It is of great pleasure to have been able to study such a collection, and I appreciate very much this privilege given me by Prof. HÓZAWA. My thanks are also due to Prof. HIRASAKA, of the Zoological Department, Taihoku Imperial University, for the loan of several important monographs and journals concerning the subject of this study.

The numerous specimens sent to me were preserved in alcohol. Beside these, there have been in my possession a number of dried specimens mostly from the same district as the former. These latter have been useful for this study, because the loops are often very well preserved in them.

The following is the list of species identified :

1. *Lingula nipponica* HAYASAKA.
2. *Hemithyris psittacea* GMELIN var. *woodwardi* ADAMS.
3. *Terebratulina* sp. indet.
4. *Terebratalia coreanica* ADAMS & REEVE.
5. *Coptothyris grayi* (DAVIDSON) subsp. *aomoriensis*, HAYASAKA.
6. *Laqueus rubellus* SOWERBY.

ments  
— of these six, *Terebratalia* and *Coptothyris* are quite common, while  
<sup>1)</sup> *Contus* and *Hemithyris* are represented by a smaller number of specimens :  
<sup>2)</sup> *P07*

<sup>3)</sup> Contributions from the Marine Biological Station, Asamushi, Aomori-ken. No. 76.



*Lingula* and *Terebratulina* are not more than three and one, respectively.

*Terebratalia coreanica* is rather wide-spread in the seas around Japan, and is also found as fossil at several places in Japan. *Coptothyris grayi* subsp. *aomoriensis* has not hitherto been recorded, but it seems to be rather common in the northern part of Japan. *Hemithyris psittacea* subsp. *woodwardi* is confined to the seas of North Japan, while *Laqueus rubellus* is found in both southern and northern Japan. For the specific determination of *Terebratulina*, more material is necessary, though the only specimen at hand greatly resembles *T. caput-serpentis*, a widely distributed species. Those are also known as fossil in the Japanese Cainozoic. The occurrence of *Lingula* from the coast of the northern part of Japan is recorded for the first time.

On the whole, the Brachiopod fauna is composed of species that are not found in the waters outside the limits of those surrounding Japan. These seem to have been in existence in this part of the world since the time of late Tertiary.

The seas around the Japanese Islands have for long been known for its very rich Brachiopod fauna. But it is worthy of note that hardly any species of these seas seem to occur in other seas. Thus, Brachiopods alone may not be of much significance for the purpose of faunal correlations.

#### DESCRIPTION OF THE SPECIES.

##### 1. *Lingula nipponica* HAYASAKA.

(Pl. I, Fig. 1; Pl. II, Fig. 1.)

? 1888 *Lingula anatina*, DAVIDSON (pars):—Trans. Linn. Soc. London, 2nd ser. (Zoology), vol. IV, pt 3, p. 206, pl. 30, fig. 6.

1931. *Lingula*, n. sp., HAYASAKA:—The Venus, vol. III, No. 1, p. 2, Fig. 1.

1931. *Lingula nipponica*, HAYASAKA:—Kwagaku (The Science), vol. I, No. 9, p. 364.

The name in use for a long time, *anatina*, was abandoned sometime ago because of the priority of LINNÉ's old name *unguis*<sup>1)</sup>. This species has been studied repeatedly by various scientists since the time of LINNÉ, in 1858<sup>2)</sup>. The characteristics of this species are, therefore, well defined in most of the papers of those scientists.

The species here under consideration have almost all the characteristics in common with *L. unguis*: the only point of difference is in the out-

<sup>1)</sup> DALL: Proc. U. S. Nat. Mus., vol. 57, p. 262. 1920.

THOMSON: Brachiopod Morphology and Genera, p. 124. 1927.

<sup>2)</sup> For literature, see DAVIDSON, DALL, THOMSON, l. c.

**Studies on the Physiology of Ciliary Movement.**  
**I. Effect of Hydrogen Ion Concentration upon the Ciliary**  
**Movement of the Gill of *Pecten*.<sup>1)</sup>**

By

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(Biological Institute, Tôhoku Imperial University, Sendai.)

(Received Nov. 5th, 1931.)

**CONTENTS.**

Introductory

Material and Method.

Experiment. —

Effect of lack of oxygen and effect of CO<sub>2</sub>

Effect of hydrochloric acid.

Effect of phosphoric acid.

Effect of acetic acid.

Addenda: — Effect of CO<sub>2</sub>.

Effect of hydrochloric acid.

General Remarks and Discussion.

Summary and Conclusion.

Literature cited.

**INTRODUCTORY.**

Since PÜTTER reviewed<sup>2)</sup> the literature on the physiology of the ciliary movement thithereto appeared, time has elapsed and much has been done by various investigators. GRAY, among the workers, undertook the integrant part of the recent advances in the knowledge of the subject, and his book<sup>3)</sup> appeared in 1928, which comprises the later results obtained since the article of PÜTTER was written.

Many problems, however, remained open to question or have arisen anew, as is always the case in science when our knowledge is advanced or the method of attack is refined.

The author will publish in a series of papers the results of the experiments upon the ciliary movement, planned from a standpoint of general

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<sup>1)</sup> Contributions from the Marine Biological Station of Asamushi, No. 77.

<sup>2)</sup> PÜTTER, 1903.

<sup>3)</sup> GRAY, 1928

physiology, hoping if possible to contribute to the advancement of the knowledge in this line.

I have determined the intracellular oxidation-reduction potential limiting the ciliary movement of the ciliated cells of the gill of *Pecten yessoensis* JAY, under the anaerobic conditions caused by the exhaustion of oxygen by their own respiratory metabolism in the closed glass vessel.

The report of the work just mentioned will appear in another place in the near future<sup>1)</sup>. In connection with this, it was necessary for us to conduct a preliminary experiment on the effect of acids, especially that of carbonic acid, upon the ciliary movement of the same material.

The present paper comprises the results of this preliminary work with various kinds of acid in different concentrations expressed in terms of pH.

As the main purpose of the present experiment was to examine the relation between the hydrogen ion concentration and the oxidation reduction potential in the ciliated cell at the stoppage of the ciliary movement, caused by the lack of oxygen or possibly by the accumulation of carbon dioxide, the experiment was not extended to so many kinds of acid.

I take this opportunity of expressing my cordial thanks to Dr. S. KOKUBO for his valuable help given me in the course of the work.

#### MATERIAL AND METHOD.

A small respiratory chamber was made by fixing a glass ring, about eight millimeters in diameter and four millimeters in width cut from glass tubing, on a slide glass with de Khotinsky cement.

The chamber was filled with sea-water and a small piece of gill tissue, a few millimeters on sides, from the ventral margin of the gill, was placed therein, then the chamber was closed with a cover slip and sealed with vaselin. The movement of the cilia could be observed from above through the cover slip under the microscope of moderate magnifications (100 x to 400 x). For the sake of convenience, observation was made on the movement of the terminal cilia at the ventral tip of the gill filament, which causes the current along the ventral margin of the gill leaf. Fresh or acidified sea-water employed as medium was coloured with pH indicators such as phenol red, brom thymol blue, brom cresol green or brom cresol purple, as the case demanded, and the pH value of the medium could be read not only at the beginning but also during the course of the experiment by comparing the colour of the medium viewed from the side of the

<sup>1)</sup> NOMURA 1932.

# CONTRIBUTIONS TO THE PHYSIOLOGY OF THE ASCARIS

## V. SURVIVAL AND RESPIRATORY EXCHANGE OF THE ASCARIS, *ASCARIS MEGALOCEPHALA* CLOQ. INTERCEPTED FROM LIGHT IN PRESENCE AND ABSENCE OF OXYGEN

By

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(With four Text-figures)

(Received March 5, 1936)

### INTRODUCTION

The usual seat of ascaris in host is the small intestine, where it is totally intercepted from light in absence of oxygen. They often wander into the stomach, and exceptionally get into the bronchi, nose, coelom, vagina, etc., where oxygen is present, but still without light. Thus the ascaris lives without any influence of light throughout its entire life without relation to the presence or absence of the oxygen, accordingly the survival and the respiratory exchange of the ascaris kept in the medium intercepted from light in the presence and the absence of oxygen is of interest.

The respiratory exchange of the ascaris has been studied by several investigators. WEINLAND (1901-1902) found that *Ascaris lumbricoides* outputs 0.4 gms. of carbon dioxide per 100 gms. of the worm in twenty-four hours in the absence of oxygen. OKABE (1932) found on the average 0.1768 mgms. of carbon dioxide per 1 gms. of *Ascaris lumbricoides* in 30 minutes in the absence of oxygen. The present author (1934) has also investigated the respiratory exchange of *Ascaris megalcephala* not only in absence of oxygen, but also in presence of oxygen and stated that the total amount of carbon dioxide produced in 24 hours at 38°C. was from 80 (female) to 200 cc. (male) per 100 gms. of the worm in the presence of oxygen, and from 20 (female) to 80 cc. (male) in the absence of it. None of these data are, however, ideal, because the influence of light which may bring about some abnormal life phenomena for the worm has been neglected.

As regards the survival of the ascaris, BUNGE found that *Ascaris lumbricoides* was able to live from 4 to 6 days in 1 per cent solution of sodium chrolide deprived of oxygen and stated that a least amount or

absence of oxygen is favourable for the life of the worm. WEINLAND (1901-1902) found that *Ascaris lumbricoides* lived from about 7 to 9 days in the solution boiled and saturated with carbon dioxide and stated that a great amount of the gas is favourable for the existence of the worm. I have (1935) found that *Ascaris megalocephala* lived long (70 to 110 hours) in Ringer's solution containing a large amount of carbon dioxide, but short-lived (20 to 50 hours) in the solution containing oxygen. These data are also obtained under the neglect of the influence of light. Thus there is no one who has studied the survival of the ascaris intercepted from light.

In the present investigation I have dealt with the carbon dioxide production and the oxygen consumption of *Ascaris megalocephala* intercepted from light in the presence and absence of oxygen from healthy condition to the point of death, as the fifth step of the investigations concerning the anaerobic changes of the ascaris, with the hope of obtaining more accurate data about the respiratory exchange of the worm, and also with the survival of the worm kept under the condition of no light to determine whether or not the total darkness is more favourable for the life of the worm.

#### MATERIALS AND METHODS

The ascarides used in my experiment were found in the small intestines of horses raised for the anatomical researches of the students of the Morioka Imperial College of Agriculture and Forestry. Some of the specimens were also collected from the Morioka Slaughter house. In this experiment only fresh, healthy worms varying in weight from about 4 to 7 gms. in the female worms and from 1 to 2 gms. in the male ones, were used.

For the determining of the amount of carbon dioxide produced in the anaerobic existence of the worm, the following medium was used; Ringer's solution was boiled in order to drive off the oxygen and then saturated with nitrogen that was purified by passing it through a pyrogalllic acid solution. Thus the solution contained no oxygen.

For the determining not only the amount of the carbon dioxide produced, but also the oxygen consumed in the aerobic existence of the worm, Ringer's solution of a known concentration of oxygen and carbon dioxide was used.

To intercept the worm from light the following method was employed :

A bottle, on the wall of which was painted black enamel, was obtained, and 500 cc. of the solution above mentioned was drawn into the bottle by means of a siphon under paraffin oil in order to prevent direct contact with the air. The aperture of the bottle was stopped after the specimens were placed separately in it. Under these conditions the worm lived for about from 25 to 60 hours.

Preceding the experiment, the specimens were kept in the dark bottle prepared by the method above mentioned for 5 hours to avoid the errors owing to not only a great amount of carbon dioxide contained in the intestinal fluid and the coelomic fluid of the worm, but also the micro-organisms attached to the worm. Then each specimen was replaced by a freshly prepared one for the experiments.

It was not necessary to stir the medium during the experiments, because a uniform distribution of the gases in the medium was facilitated by the continuous peristaltic movement of the worm. By placing the bottle in a thermostat the temperature was kept constant at 38°C. which is the normal temperature of the horse.

To determine the decrease and the increase of the oxygen and the carbon dioxide in the medium, caused by the respiration of the worm, Van Slyke's method was used. In my experiment, 2 cc. of the medium at fixed intervals were used for analysis.

1 N. lactic acid was used for freeing the carbon dioxide from the medium which contain carbonate.

The reagent used for the absorption of the oxygen was alkaline pyrogallol, while for the absorption of the carbon dioxide a 5 N. sodium hydroxide solution was used.

#### RESULT OF THE EXPERIMENT

I have preliminarily ascertained that the usual seat of *Ascaris mega-locephala* is totally intercepted from light. The method employed was as follows: The abdominal wall of the horse, 4 cm. in thickness, was taken, and the plate was wrapped in it and exposed to a globe of 200 candle-power at the distance of 50 cm. for 30 minutes in the dark room or to the light in the open air for the same duration.

*1. Result Obtained for the Survival of Ascaris megalocephala Intercepted from Light in the Presence and the Absence of Oxygen.*

I have compared the survival of *Ascaris megalocephala* intercepted from light with that not intercepted from it. The result is as follows: In Table 1 are given the results obtained in the solution intercepted from light and those not intercepted from it in the presence of oxygen at 38°C., in Table 2 the results in the solution deprived of oxygen and saturated with carbon dioxide at 38°C. and in Table 3 the results in the solution derived off the oxygen and saturated with pure nitrogen.

TABLE 1.

*Survival of the ascaris in the medium intercepted from light and those not intercepted from it in the presence of oxygen.*

Survival in the light		Survival in the dark	
Body weight in gms	Time in hours	Body weight in gms.	Time in hours
1.15 ♂	32	1.80 ♂	23
1.80 "	27	1.90 "	28
1.85 "	35	1.95 "	35
1.95 "	42	1.95 "	40
2.00 "	30	2.00 "	44
2.10 "	45	2.10 "	26
2.10 "	40		
Average 1.85 "	36	Average 1.95 "	33
		3.95 ♀	28
1.85 ♀	35	4.10 "	37
5.00 "	10	4.45 "	29
5.10 "	27	5.20 "	38
5.20 "	29	6.00 "	40
5.20 "	38	6.00 "	33
6.45 "	44	7.15 "	42
Average 5.30 "	35	Average 5.31 "	35

In the presence of oxygen, as will be seen in Table 1, the duration of life of *Ascaris megalocephala* intercepted from light is approximately the same as that not intercepted from it, showing on the average about 35 hours in both cases. As will be seen in Table 2, a similar relation is also observed in the medium saturated with carbon dioxide after deprived of oxygen. In this case, however, the duration of life is greatly long, being about 70 hours in the male worms and about 90 hours in the female ones without relation to whether the light is given or not.

TABLE 2.

*Survival of the ascaris intercepted from light and that not intercepted from it, when the medium was saturated with carbon dioxide after deprived of oxygen.*

Not intercepted from light		Intercepted from light	
Body weight in gms.	Time in hours	Body weight in gms.	Survival in hours
0.85 ♂	85	1.00 ♂	88
0.90 ..	90	1.20 ..	72
1.20 ..	74	1.25 ..	68
1.35 ..	61	1.45 ..	75
1.80 ..	65	1.45 ..	64
1.95 ..	69		
Average 1.34 ..	74	Average 1.23 ..	73
2.70 ♀	77	3.95 ♀	90
3.10 .	66	4.20 ..	95
2.90 ..	65	4.40 ..	89
3.26 ..	115	4.85 ..	67
3.70 ..	90	5.15 ..	101
3.90 ..	102	3.30 ..	99
4.05 ..	96	Average 4.61 ..	90
Average 3.37 ..	86		

TABLE 3.

*Survival of the ascaris intercepted from light and that not intercepted from it, when the medium was saturated with pure nitrogen after deprived of oxygen.*

Not intercepted from light		Intercepted from light	
Body weight in gms.	Survival in hours	Body weight in gms.	Survival in hours
1.00 ♂	32	0.50 ♂	43
1.00 ..	24	1.10 ..	48
1.75 ..	29	1.25 ..	39
1.80 ..	37	1.80 ..	44
1.95 ..	26	1.90 ..	47
Average 1.50 ..	30	Average 1.31 ..	44
4.50 ♀	24	4.00 ♀	50
4.65 ..	22	4.25 ..	48
4.80 ..	32	4.35 ..	45
5.00 ..	26	5.30 ..	52
6.30 ..	29	5.75 ..	50
6.55 ..	21	7.00 ..	46
Average 5.47 ..	26	Average 5.11 ..	49



In the solution saturated with nitrogen after deprived of oxygen, as will be seen in Table 3, a remarkable variation is found between the survival in the dark and in the light: namely the worm lived about 46 hours in the dark, but only about 25 hours in the light.

As regards the survival of *Ascaris megalocephala* in the light, I have already stated in the third report of this investigation (1935) that the absence of oxygen is favourable for the life of the worm from the fact that the worm lives longer in Ringer's solution saturated with carbon dioxide than in the solution saturated with oxygen. But it is noted from the results above obtained that the absence of oxygen is not necessarily favourable for the life of the worm; namely the worm in the light is most short-lived in the medium saturated with pure nitrogen after deprived of oxygen. This shows that the duration of life of *Ascaris megalocephala* varies with the kind of gases dissolved in the medium, the most favourable gas being carbon dioxide, the next one oxygen and the last one nitrogen.

At any rate, it is noted from the results above obtained that the darkness does not become favourable for the life of *Ascaris megalocephala* in both the medium saturated with carbon dioxide and that supplied with oxygen, but becomes favourable for the worm in the medium saturated with pure nitrogen after deprived of oxygen. This suggests that the light does not acts as toxic for the life of the worm in the medium containing suitable gas, but acts as toxic for the worm in the medium containing unsuitable gas. To support the view just stated I noticed that the duration of life of the worm in the solution intercepted from light in the presence of carbon dioxide or oxygen is almost the same as that not intercepted from light, but that of the worm in the medium intercepted from light in the presence of nitrogen is twice as much as that not intercepted from it as mentioned already.

## 2. Results Obtained for the Respiratory Exchange of *Ascaris megalocephala* Intercepted from Light in Absence of Oxygen.

Since it was found in a previous section that Ringer's solution saturated with pure nitrogen after deprived of oxygen was favourable for the life of *Ascaris megalocephala* intercepted from light as above mentioned, the solution was used as a medium for determining the carbon dioxide production of the worm intercepted from light in the absence of oxygen.

Preceding the experiment each specimen was kept in the solution for five hours at the temperature to which it was to be subjected to avoid the errors owing to a great amount of carbon dioxide contained in the intestinal fluid and the coelomic fluid of the worm.

The results obtained are as follows: The data obtained at 38°C. are given in Table 4, and are shown graphically in Figure 1. In Table 5 are given the results obtained at low temperature (20–25°C).

TABLE 4.

*Carbon dioxide evolved by the worm intercepted from light in absence of oxygen.*

Body weight in gms.	Time in hours	CO <sub>2</sub> content in Vol. %	CO <sub>2</sub> evolved in Vol. %
6.40 ♀	0	5.248	
	1	5.312	0.064
	2	5.270	0.022
	4	5.408	0.160
	6	5.600	0.352
	8	6.208	0.960
	15	6.592	1.244
	24	6.464	1.216
	28	6.496	1.248
	32	6.336	1.068
	46	6.688	1.442
	50	6.752	1.504
3.00 ♀	0	5.404	
	1	5.408	0.004
	2	5.440	0.036
	4	5.472	0.068
	6	5.600	0.196
	10	5.984	0.580
	15	6.144	0.740
	20	6.150	0.746
	24	6.208	0.804
	48	6.260	0.864
1.95 ♂	0	5.056	
	1	5.062	0.006
	2	5.120	0.064
	4	5.104	0.048
	8	5.344	0.288
	10	5.536	0.480
	15	5.668	0.608
	19	5.856	0.800
	24	5.952	0.896
	30	5.824	0.768
	42	5.888	0.832

TABLE 5.

*Carbon dioxide evolved by the worm intercepted from light  
in absence of oxygen (20–25°C).*

Body weight in gms.	Time in hours	CO <sub>2</sub> content in Vol. %	CO <sub>2</sub> evolved in Vol. %
5.70 ♀	0	4.960	
	18	5.408	0.448
	24	5.400	0.440
	44	5.728	0.768
	68	6.016	1.056
	93	6.816	1.756
	117	6.912	1.952
	141	7.206	2.246
	165	6.913	1.953
	186	6.592	1.632
1.50 ♂	0	5.184	
	24	6.080	0.896
	44	6.336	1.152
	66	6.432	1.248
	93	6.624	1.440
	116	6.400	1.216
	140	6.752	1.568
	188	6.976	1.792

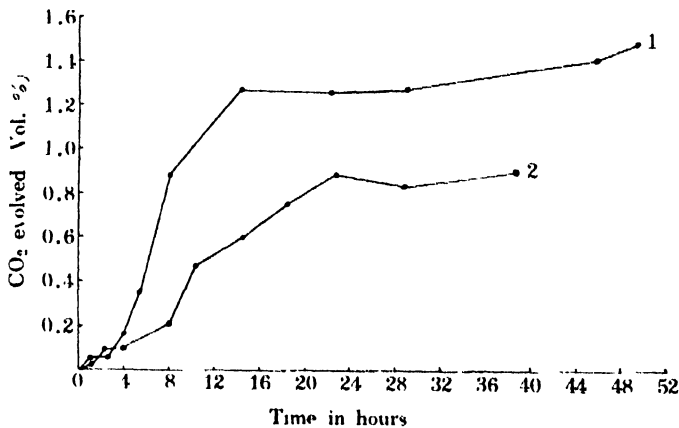


Fig. 1. Showing the changes in CO<sub>2</sub> evolved in the medium intercepted from light in absence of oxygen.

1. Adult female 6.40 g.) 2. Adult male 1.95 g.)

As is shown in Table 4 and Figure 1, no carbon dioxide production occurs during the first few hours of the experiment at 38°C. In about from 2 to 4 hours, however, its production begins. This continues for about 10 hours; the succeeding period shows almost no more production

of it until approaching the point of death.

As is shown in Table 5, a similar relation, as far as the carbon dioxide production is concerned, is also obtained in the experiment at low temperature. In this case, the survival of the worm is about 3 times as much as at 38°C.

In a previous paper (1934), I have already stated that *Ascaris megalocephala* placed in the light can live without oxygen and no carbon dioxide production occurs, except the duration of a few hours in the middle of the experiment. A same tendency is also observed in the present investigation in the dark as mentioned above, though both the duration of carbon dioxide production and that of no more production of it are greatly longer in the dark than in the light. Accordingly the survival of the worm in the dark is twice as much as that in the light, showing that the darkness is favourable for the life of the worm.

As regards the fact that almost no carbon dioxide production occurs during the first 2-4 hours of the experiment, it seems to be related with the production of lactic acid. Indeed, I have already stated in a previous paper (1935) that lactic acid production occurs during the first several hours of the experiment in the light. A similar relation perhaps occurs in the present investigation in the dark. To support the view just stated, I noticed that the pH value in the solution, in which the worm was kept, very likely became acidic during the first several hours of the experiment, as will be seen in Figure 4, notwithstanding the carbon dioxide production was not observed. This suggests that the lactic acid are formed from glycogen by a fermentation process. If the relation just stated is granted, it also becomes highly probable that the lactic acid production continues longer in the light than in the dark from the fact that the duration from the beginning of the experiment to the point at which the carbon dioxide production begins is longer in the worm intercepted from light than that not intercepted from light.

As to the carbon dioxide produced for a time in the course of the experiment, I notice the following relation: first, since a fermentation process undergoes, by which the glycogen split into the intermediate substances, such as valeric acid and propionic acid as already stated in the fourth report of this investigation, the corresponding amount of carbon dioxide would be produced from the glycogen, second, since in about from 2 to 4 hours of the experiment the medium used becomes acidic owing to the production of the intermediate substances, it is highly probable that some amount of carbon dioxide would be produced from the

carbonate which exists in the worm.

In the second report of this investigation (1934) I have described that *Ascaris megalocephala* not intercepted from light in the absence and the presence of oxygen can live without carbon dioxide production after about 24 hours of the survival of the worm. A similar relation is also observed in the present investigation in the worm intercepted from light. In this case, however, the duration is about 30 hours, being about twice as much as that obtained in the worm not intercepted from light. It seems to me that during this period a special kind of fermentation process undergoes, by which the glycogen split into the intermediate substances and the carbon dioxide, but at once it would be used up for reduction process. To support the view just stated I noticed the following relation: first, as has already stated in the third report of this investigation, *Ascaris megalocephala* not intercepted from light in the absence and the presence of oxygen consumed about 60 per cent of the total glycogen contained in the worm within 24 hours of the survival, but afterwards consumed only a minute amount of it, and even at the point of death about 30 per cent of the total glycogen was remained, second, as will be seen in Figure 4, after 15 hours of the experiment pH value in the solution was almost unchanged. The reduction process just stated perhaps continues longer in the worm intercepted from light than not intercepted from it from the fact that the duration of no carbon dioxide production is longer in the former than in the latter.

### 3. Results Obtained for the Respiratory Exchange of the Worm Intercepted from Light in Presence of Oxygen.

The results obtained at 38°C. are given in Table 6, and are shown graphically in Figures 2 and 3. In Table 7 are given the results obtained at 20°-25°C.

TABLE 6.

*Carbon dioxide evolved and oxygen consumed by the worm intercepted from light in presence of oxygen.*

Body weight in gms.	Time in hours	O <sub>2</sub> content in Vol. %	O <sub>2</sub> consumed in Vol. %	CO <sub>2</sub> content in Vol. %	CO <sub>2</sub> evolved in Vol. %	Respiratory quotient
6.00 ♀	0	0.452		5.152		
	1	0.437	0.015	5.216	0.060	4.027
	2	0.480		5.200	0.048	
	4	0.288	0.164	5.664	0.512	3.122

Body weight in gms.	Time in hours	O <sub>2</sub> content in Vol. %	O <sub>2</sub> consumed in Vol. %	CO <sub>2</sub> content in Vol. %	CO <sub>2</sub> evolved in Vol. %	Respiratory quotient
6.00 ♀	5	0.224	0.228	5.856	0.704	3.088
	7	0.128	0.324	6.208	1.056	3.259
	10	0.096	0.356	6.432	1.280	3.598
	15	0.078	0.374	6.496	1.334	3.567
	20	0.109	0.343	6.624	1.572	4.583
	24	0.047	0.405	6.514	1.362	3.363
	27	0.064	0.388	6.528	1.376	3.546
3.05 ♀	0	0.480		5.248		
	1	0.432	0.048	5.280	0.032	0.667
	2	0.496		5.280	0.032	
	4	0.400	0.080	5.328	0.080	1.000
	6	0.250	0.230	5.440	0.192	0.835
	12	0.156	0.324	5.696	0.448	1.383
	15	0.062	0.418	6.080	0.832	1.990
	20	0.031	0.449	6.112	0.864	1.924
	22	0.047	0.433	6.016	0.768	1.774
	26	0.045	0.435	6.280	0.960	2.207
1.75 ♂	0	0.499		4.928		
	1	0.484	0.015	4.764		
	2	0.499		4.960	0.032	
	4	0.468	0.031	5.024	0.096	3.097
	6	0.312	0.187	5.120	0.292	1.561
	8	0.249	0.249	5.280	0.352	1.114
	12	0.156	0.343	5.632	0.704	2.052
	15	0.096	0.403	5.760	0.832	2.065
	20	0.078	0.421	5.824	0.896	2.128
	24	0.062	0.437	5.824	0.896	2.050
	27	0.062	0.437	5.856	0.928	2.124

TABLE 7.

*Carbon dioxide evolved and oxygen consumed by the worm  
intercepted from light in presence of oxygen (20-25°C).*

Body weight in gms.	Time in hours	O <sub>2</sub> content in Vol. %	O <sub>2</sub> consumed in Vol. %	CO <sub>2</sub> content in Vol. %	CO <sub>2</sub> evolved in Vol. %	Respiratory quotient
5.05 ♀	0	0.749		5.280		
	8	0.686	0.163	5.344	0.064	0.396
	18	0.468	0.281	5.824	0.544	1.936
	24	0.156	0.593	6.086	0.800	1.349
	49	0.062	0.687	6.432	1.152	1.677
	69	0.093	0.656	6.496	1.196	1.823
	92	0.078	0.671	7.072	1.792	2.671
	110	0.031	0.718	7.136	1.856	2.585
1.10 ♂	0	0.702		5.440		
	18	0.406	0.296	5.504	0.064	0.216
	24	0.421	0.281	5.796	0.356	1.267
	44	0.094	0.608	5.974	0.534	0.878
	66	0.094	0.608	6.080	0.640	1.053
	93	0.047	0.655	5.824	0.384	0.586
	116	0.062	0.640	6.016	0.576	0.900
	140	0.062	0.640	6.368	0.928	1.450

As will be seen in Table 6, Figures 2 and 3, almost no oxygen consumption nor carbon dioxide production occurs during the first few hours of the experiment. In about from 2 to 4 hours, however, the oxygen consumption begins. This continues at almost the same rate (about 11 cc.

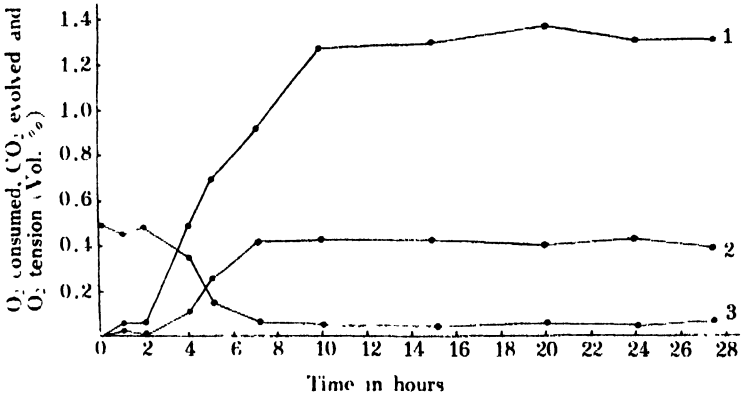


Fig. 2 Showing the changes in O<sub>2</sub> consumed and CO<sub>2</sub> evolved in the medium intercepted from light in presence of oxygen (Adult female).

1 CO<sub>2</sub> evolved 2 O<sub>2</sub> consumed 3 O<sub>2</sub> tension

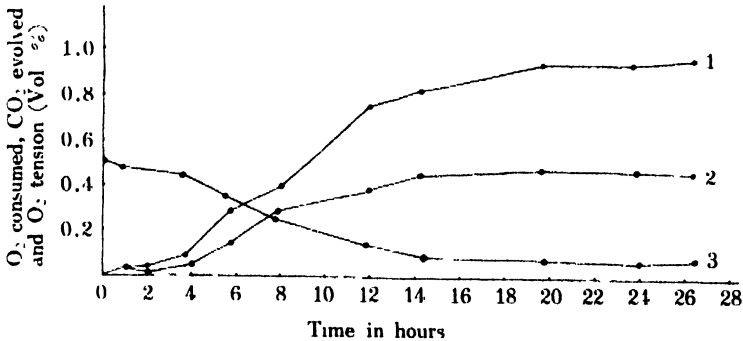


Fig. 3 Showing the changes in O<sub>2</sub> consumed and CO<sub>2</sub> evolved in the medium intercepted from light in presence of oxygen (Adult male).

1 CO<sub>2</sub> evolved 2 O<sub>2</sub> consumed. 3 O<sub>2</sub> tension.

per 100 gms. of the worm in 2 hours) during several hours afterwards until the oxygen tension in the medium becomes about 0.06, indicating that the rate of oxygen consumption is independent of the oxygen tension in the medium as in the aerobic animals. It is also to be noted that during this period just mentioned the carbon dioxide production occurs

in the same manner as during the experiment in absence of oxygen, producing about 15 cc. per 100 gms. of the worm in 2 hours. Almost no more production of it occurs after about 15 hours of the experiment until approaching the point of death.

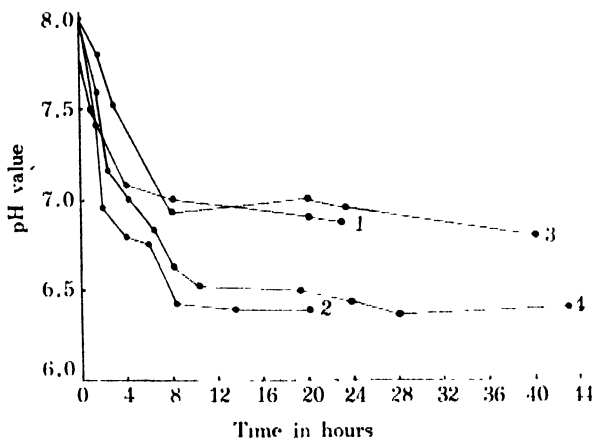


Fig 4. Showing the changes in pH value of the solution intercepted from light in presence and absence of oxygen

- 1 In presence of oxygen (Adult male).
- 2 " " (Adult female)
- 3 In absence of oxygen (Adult male)
- 4 " " Adult female).

As is shown in Table 7, a similar relation, as far as the oxygen consumption and the carbon dioxide production is concerned, is also obtained in the experiment at 20°-25°C. In this case, the survival of the worm is about 3 times as much as at 38°C., accordingly both the rate of oxygen consumption and that of carbon dioxide production are much smaller than those at 38°C.

There is a remarkable variation in the respiratory exchange between the small ascaris and the large one; the carbon dioxide production is approximately parallel to the oxygen consumption in the small one, but not in the large one, in which the amount of carbon dioxide produced exceeds that of the oxygen consumed and the larger the worm, the more the excess of it is observed.

The result obtained from the above experiment seems to indicate that since ascarides normally exist in the absence of oxygen they find it very difficult to take it at first, but soon become adapted to its presence and oxydative reaction takes place. It should be considered, however, that



most amount of carbon dioxide is produced from glycogen by a fermentation process and only a part of it by an oxydative reaction. To support

TABLE 8.

*Comparison of the amount of carbon dioxide produced or oxygen consumed by the worm intercepted from light with those by that not intercepted from it.*

Parasite	Temperature	Time in hours	Cases	O <sub>2</sub> consumption per 100 gms. in cc	CO <sub>2</sub> output per 100 gms. in cc.	Respiratory quotient	Investigators
<i>Ascaris megalocephala</i> ♂	16°-19°	44	Intercepted from light in absence of oxygen	180	181	1.005	TORYU
" ♀	"	"	"	61	103	1.689	
" ♂	38°	24	"	241	230	0.954	
" Small ♀	"	"	"	79	107	1.354	
" Large ♀	"	"	"	30	102	3.400	
" ♂	16°-19°	44	Not intercepted from light in presence of oxygen	135	124	0.911	
" ♀	"	"	"	57	81	1.432	
" ♂	38°	24	"	245	195	0.796	
" Small ♀	"	"	"	92	68	0.739	
" Large ♀	"	"	"	29	84	2.896	
" ♂	20-25°	44	Intercepted from light in absence of oxygen		224		
" ♀	"	"	"		60		
" ♂	"	"	"		254		
" Small ♀	"	"	"		120		
" Large ♀	"	"	"		85		
" ♂	"	"	Not intercepted from light in absence of oxygen		75		
" Small ♀	"	"	"		58		
" Large ♀	"	"	"		20		
"	"	"	"		204		WEINLAND
<i>Ascaris lumbricoides</i>	"	30 min.	"		0.1768 (gms.)		OKABE
<i>Filalia equina</i>	38°	24	Not intercepted from light in presence of oxygen	625	650	1.040	TORYU

the view just stated I noticed in the fourth report of this investigation that *Ascaris megalocephala* produces the intermediate substances, such as valeric acid, propionic acid and lactic acid without relation to whether the oxygen is present or not in the medium; namely even when the medium was saturated with oxygen throughout the entire survival of the worm, the intermediate substances were also found, though the amount of it was somewhat less than in the medium deprived of oxygen, and furthermore, as will be seen in Figure 4, the changes of pH value in the solution, in which the worm was kept, is almost the same in both the presence and the absence of oxygen.

To compare the respiratory exchange of *Ascaris megalocephala* intercepted from light with that not intercepted from it, the total amount of oxygen consumed and carbon dioxide produced in 24 hours in the both cases were calculated. The result is given in Table 8. In the same table are also given the data of several other parasites for comparison with that of *Ascaris megalocephala*.

As will be seen in Table 8, practically no differences are found in the production of carbon dioxide in both the worm intercepted from light and that not intercepted from it in the presence of oxygen; but remarkable differences are found in the absence of oxygen, in which greatly higher amount of carbon dioxide is found in the worm intercepted from light than in that not intercepted from it. This shows that the fermentation of glycogen in *Ascaris megalocephala* proceeds without relation to light in the presence of oxygen, but not in the absence of oxygen, in which the fermentation of glycogen is more active in the dark than in the light. It is also to be noted in the same table that the production of carbon dioxide in *Ascaris megalocephala* is much less than that found for *Ascaris lumbricoides* by WEINLAND and OKABE and for *Filaria equina* by the present author. The variations just stated are probably due to the specificity of the worm used and technique employed on one hand, and to the natures of their respiration owing to the living place of the worm on the other hand.

#### SUMMARY

The results obtained in this investigation may be summarized as follows:

1. For the life of *Ascaris megalocephala*, carbon dioxide is favourable without relation to whether the worm is intercepted from light or not; namely when the worm was placed in Ringer's solution saturated with carbon dioxide after deprived of oxygen and intercepted from light its

survival was from about 70 to 100 hours, showing much the same order as that found in the light.

2. For the life of *Ascaris megalocephala*, nitrogen is favourable only in the dark; the worm lived from about 40 to 50 hours in Ringer's solution saturated with pure nitrogen after deprived of oxygen and intercepted from light, but only from about 20 to 30 hours in the solution not intercepted from light.

3. A supply of oxygen shortens the life of *Ascaris megalocephala* without relation to whether the worm is intercepted from light or not; namely when the worm was placed in Ringer's solution containing oxygen and intercepted from light its survival was from about 20 to 40 hours, showing much the same order as that found in the worm not intercepted from light.

4. The respiratory exchange of *Ascaris megalocephala* intercepted from light in presence of oxygen almost agreed with that not intercepted from it with the following results: first, when the worm were placed in Ringer's solution containing oxygen and intercepted from light they consumed the oxygen until the tension in the medium became about 0.03; second, carbon dioxide production exceeded the oxygen consumption, showing not only an oxidative reaction, but also a fermentation process.

5. Remarkable variations were found in the amount of carbon dioxide produced in both the worm intercepted from light and that not intercepted from it in the absence of oxygen; namely the total amount of carbon dioxide produced in 24 hours at 38°C. was from 80 (female) to 250 (male) per 100 gms. of the worm intercepted from light in absence of oxygen, but only from 20 (female) to 80 cc. (male) in the worm not intercepted from light in absence of oxygen.

6. Almost no carbon dioxide production nor oxygen consumption occurred during the first and last several hours of the experiment, suggesting lactic acid production from glycogen by a fermentation process in the first period of survival, but a reduction process in the last period of survival.

7. The pH value of the solution used showed a great decrease within several hours of the experiment, but afterwards almost unchanged, suggesting the same relation as mentioned above.

Before leaving the subject, I wish to express my hearty thanks to Dr. S. HATAI for his valuable suggestions and criticism throughout the entire course of this work.

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# REPORT OF THE BIOLOGICAL SURVEY OF MUTSU BAY\*

## 30. NOTES ON THE PROTOZOAN FAUNA OF MUTSU BAY

### III. SUBGENUS *PROTOPERIDINIUM*: GENUS *PERIDINIUM*

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(With fifty figures)

(Received February 5, 1936)

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#### PREFACE

Present paper is intended to describe a part of my investigation on *Protoperidinium*, a subgenus of *Peridinium* of Mutsu Bay, including three groups and seven species. The materials upon which this paper is written are those collected by Dr. S. KOKUBO during January—April, 1925–1927.

Sincere gratitude is here expressed to the SAITO Hoon-kai for publishing this paper. And I shall like to express here my hearty thanks to Prof. S. HATAI and also Prof. S. HOZAWA for their warmhearted help extended to me to arrange this manuscript for publication, to Assistant Prof. S. KOKUBO for collecting and leaving the materials to me, and to Dr. T. KABURAKI, Professor of Tokyo Imperial University for the privilege of using a room in the Institute where the present paper is written. My

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\* A contribution from the Marine Biological Station, Aomori-ken. No. 134.

hearty thanks also should be offered to late Dr. K. OKAMURA for his kindness in placing all his literatures of Dinoflagellata at my disposal.

### Subgenus *Protooperidinium* BERGH

Early investigators divided the genus into two groups, *Protooperidinium* and *Euperidinium*, and to the former was given the following definition by PAULSEN (1908 p. 41). "Querfurche rechts drehend. Keine hohle Antapical Hörner, sondern oft solid Stacheln vorhanden." But later workers all followed JÖRGENSEN's system which emphasized the mode of the combinations of the ventral and the dorsal plate patterns of the epitheca. Recognizing the existence of close relations between the general features of the ventral area and its plate pattern on the one hand, and the type of the hypotheca other than the ventral area on the other hand, and finding some diversities in the type of the ventral area and constancy in the structural relations of this area, I feel constrained to readopt here a part of the oldest system somewhat in a modified style in subdividing the genus. And the subgenus *Protooperidinium* may be defined as followings according to my observation.

The body is globular, pyriform or rhombic with or without faint antapical indentation. The hypotheca is hemispherical without antapical horn. The girdle is circular or ascending. The antapical spine is wholly absent in some of the globular or lenticular species, but in others, one or generally two are found in the posterior region immediately by or at a little distance from the posterior end of the ventral area, rising directly from the body wall without forming distinct basal hollow horn or protuberance of the wall. The slightly subsided ventral area is narrow and elongated posteriorly, without forming deep groove as a whole. It consists of four plates and the ventral or longitudinal furrow s. str. is restricted in the region occupied by the left and the anterior plates and further extends posteriorly, in some cases, into the posterior plate. The posterior plate is small and does not expand laterally beyond the median plates and anteriorly not beyond the postmargin of the left plate. The left side list of the ventral area and the flagellar fin are the two ventral projections of the hypotheca, forming a sheath for the basal parts of the flagella. The left antapical spine stands always immediately outside the left anterior end of the posterior plate, while the right at outside the right posterior corner of the plate or at a little distance from there on the right antapical plate. These spines are either wingless or buttressed by wings,

and in some cases, connected directly or indirectly with the side list of the ventral area.

In this subgenus will be included JÖRGENSEN's or PAULSEN's (1931) following sections, Humilia, Pyriformia and a part of Tabulata and Pellucida. And the species of this subgenus may be grouped in the following six groups or sections according to the structural relations of the ventral area and its appendages such as spines and fins.

- I. Group Globula.
- II. Group Monacantha.
- III. Group Humilia.
- IV. Group Rosea.
- V. Group Pyriformia.
- VI. Group Pellucida.

And in this paper three of the six, Monacantha, Humilia and Pyriformia, are considered. The two groups, Globula and Pellucida, shall be described in a later paper. The Rosea group is distinguished arbitrarily for some doubtful species with characteristic ascending girdle and removed antapical spine, and whether it is to be regarded as a compact and distinct group or to be included in the Humilia, future investigations will reveal.

#### I. GROUP MONACANTHA

This group is characterized by the possession of a single antapical spine at a short distance from the ventral area and the distinct right handed girdle.

The body is flattened from above downwards in lense- or cake-shaped and contracted distally to a minute apical horn. The girdle forms a distinctly ascending spiral. The ventral area has a relatively narrow left side list with or without ribs. And this is the sole process in the hypotheca, except the minute right antapical spine which lies at a short distance from the ventral area on the right antapical plate. The left antapical spine is absent.

The ventral plate pattern of epitheca is "meta" and the first apical plate is very oblique, much reducing the first preingular to a minute plate. The middle intercalary plate 2a is quadrangular.

To this group is to be included two species as valid, *P. monacantha* BROCH (= *P. complanatum* MEUNIER, not KARSTEN) and *P. subcurvipes* LEBOUR. They are reported from the north atlantic and the arctic seas. And the following four doubtful species and one subspecies also have some relations with this group.



*Peridinium cerasus* PAULSEN (PAULSEN 1908, p. 43, Fig. 52; 1911, p. 307, Fig. 5; PETERS 1928, p. 45-47, Fig. 12 a-d: ? KISSELEV 1928, p. 39, Fig. 4, non LEBOUR 1925).

*P. finlandicum* PAULSEN (PAULSEN 1908, 2, p. 51, Fig. 65).

*P. roseum* PAULSEN (PAULSEN 1908, p. 44, Fig. 53).

*P. turgidum* MEUNIER (SABELINA 1930, Fig. 4 f).

*P. turgidum* v. *kariarum* SABELINA (1930, Fig. 4 a-e).

These may be the species to be included in the group *Rosea*. But our grouping of these species and their inclusion in the *Monacantha* group is an arbitrary assignment, based primarily on the presence of the separated right antapical spine coupled with distinct ascending girdle, straight extension of the anterior part of the ventral area and narrowing of its posterior end. These species show some transitional features between the two groups, *Monacantha* and *Humilia*.

This group is most closely related to the group *Humilia*, but can be distinguished from it by its smaller posterior plate, lesser degree of the girdle displacement coupled with broad postcingular row of plates, and the presence of the removed single spine.

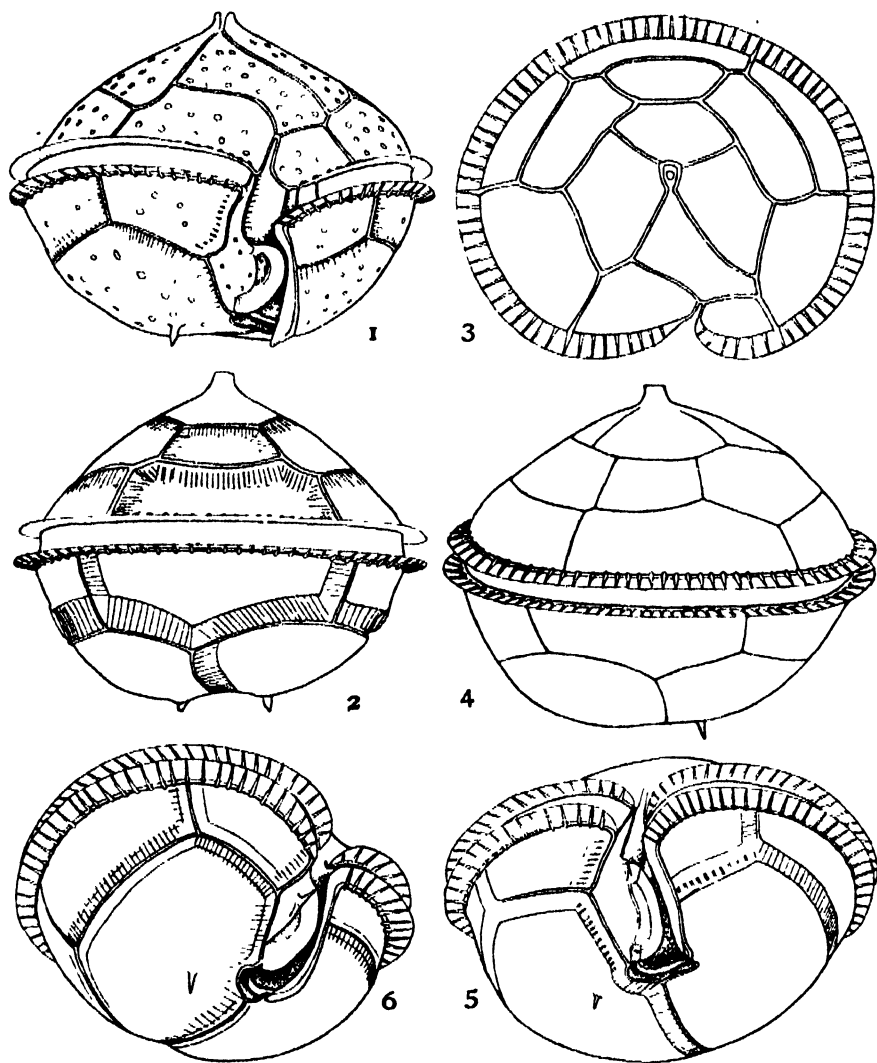
#### 1. *P. subcurvipes* LEBOUR (?)

(Figs. 1-8)

LEBOUR 1925, p. 133, Fig. 3, Pl. 17.

This species is characterized by its separated antapical spine, its rounded but somewhat flattened body, the small posterior plate and rightwards triangular expansion of the right plate.

The body is slightly flattened in cake-shaped and contracts distally to a minute but distinctly differentiated apical horn (Figs. 1, 2, 4). And there is a slight ventral median indentation of the body, making the cross section of the body at the girdle a broadly reniform, and further extends posteriorly to the antapex (Fig. 6). The girdle divides the body into two equal parts, forming itself a distinct ascending spiral with terminal arches, and is displaced distally 0.5-1 girdle width. Its side lists are well ribbed and the posterior component extends posteriorly along the median margin of the proximal postcingular plate, forming the left side list of the ventral area (Fig. 1). The antapical spine is short, standing at a distance from the postmargin of the ventral area, springing from the right antapical plate. It is straight in most cases but rarely curved distally to the right.



*Peridinium subcurvipes* LEBOUR

Fig. 1. Ventral view of body showing general feature of ventral area and surface marking of thecal plate ( $\times 600$ ).

Fig. 2. Dorsal view ( $\times 600$ ).

Fig. 3. Apical view of epitheca ( $\times 600$ ).

Fig. 4. Dorsal view of another specimen showing asymmetrical dorsal plate pattern ( $\times 600$ ).

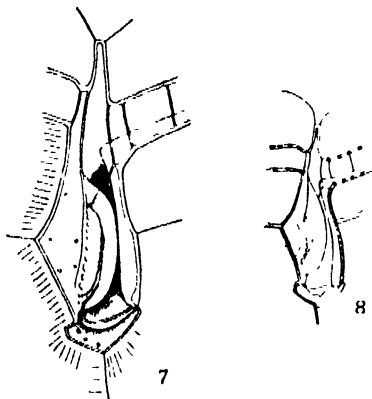
Fig. 5. Oblique antapical view of hypotheca, showing posterior portion of ventral area together with flagellar fin, flagellar trough, longitudinal furrow s. str. ( $\times 600$ ).

Fig. 6. Right oblique antapical view of hypotheca, showing posterior median indentation of body ( $\times 600$ ).

The ventral plate pattern of epitheca is "meta" and the small mid-dorsal intercalary 2a is quadrangular. There occur two types in the dorsal plate pattern concerning the plate 2a, the one asymmetrical (Fig. 4) and the other symmetrical (Fig. 2). The irregular midventral apical is very oblique with its left half slouches down to the girdle, reducing the the first precingular plate. It does not extend to the girdle but is removed from it by a long anterior extension of the anterior plate. And another three large apicals gather to form a smooth trapezoidal dorsal outer-contour (Fig. 3).

The apical closing platelet is also plainly detected in this species. The postcingular row is narrow and the antapical plates are exceedingly large, forming by far the greater portion of the hypotheca.

The transitional plate is narrow and lies transversely at the proximal end of the girdle, and the first cingular plate is small rectangular. The ventral area is short, not extending to the center of the hypotheca and irregular in contour, expanding rightwards with a bluntly pointed middle part, while its left side runs straight. It tapers anteriorly to a narrow extension, which indents the epitheca, and its posteriormost part is constricted from the rest at the anterior margin of the posterior plate, the constriction being particularly prominent on the right (Fig. 7). The area is, accordingly, narrow and lies slightly oblique in its anterior and broad and meridional in its posterior parts. A slender and somewhat oblique anterior extension of the anterior plate deeply indents the epitheca, intervening between the two terminal precingular plates,



*Peridinium subcurvipes* LEBOUR

Fig. 7 Ventral view of ventral area ( $\times 1500$ )

Fig. 8 Ventral view of somewhat modified ventral area of another specimen with elongated flagellar fin and well ribbed left side list. The ribs are illustrated only basally ( $\times 600$ )

and thus removing the ventral apical plate from the girdle (Figs. 1, 7 and 8). The elongated and irregularly triangular right plate, widest and bluntly pointed at the postcingular-antapical suture, extends anteriorly to the epitheca, and on its posterior left margin bears a broad hemicircular flagellar fin, which has a crowded line of minute ribs along its base.

The broad and somewhat straightened postmargin of the small and irregular left plate protrudes over the anterior margin of the posterior plate, forming a narrow list or lip (Figs. 1, 5-8). The flagellar pore is elongated reniform, lying somewhat obliquely and the short and distinct flagellar trough lies immediately inside the posterior right-hand constriction of the ventral area. The longitudinal furrow s. str. is restricted in the region occupied by the three components of the ventral area excluding the right plate (Fig. 7). Together with the broad flagellar fin, the left side list, which is restricted basally only along the left margin of the left plate, guard the longitudinal furrow, and this, together with the absence of any other wing or list in or outside the ventral area and the restriction of the furrow, suggest a probable functional differentiation of the right plate from the other three, concerning water current caused by the flagella. The left side list is hyaline in most cases, but rarely provided with distinct ribs of regular intervals as those of the cingular list, and in the latter case the flagellar fin extends further anteriorly to the height corresponding to the distal end of the posterior cingular list (Fig. 8).

The thecal plate is hyaline and has sparingly scattered, circular minute thickenings, each with a minute central pore (Fig. 1). The seemingly differentiated right plate is also porulated very sparingly along its outer and inner margins (Fig. 7), and there are three or four pores to be seen on the posterior plate. And I have found, on the other hand, a few specimens with fine meshes on the thecal plate.

Dimensions: Body length including the apex 56-60  $\mu$ , transverse diameter 62-73  $\mu$ , dorso-ventral diameter 50-55  $\mu$ , width of girdle 5-5.5  $\mu$ .

The antapical spine of LEBOUR's species seems to be less removed and more evidently curved than that of our species. But this may be an individual or local variation.

## II. GROUP HUMILIA

### Section Humilia JÖRGENSEN

Based mainly upon the combination of ventral and dorsal plate patterns of epitheca, JÖRGENSEN (1912) grouped those *P. ovatum*, *P. roseum*, *P. decipiens* and others under his sixth section Humilia. But recently PAULSEN (1931) removed *P. ovatum* from the section to the Pyriformia. This difference of interpretation between them is probably due to a lack of accurate knowledge on the skeletal morphology of the

genus *Peridinium* at that time, to establish a sharp distinction between this and the other groups.

A revision of old literatures, together with the data obtained from our own observation on the winter plankton of Asamushi, necessitated me to rearrange the definition of the group Humilia as following.

The globular or slightly compressed body has a distinct apical horn and two antapical spines, the latter is wingless in some cases, but often buttressed with single, or rarely two wings. The ventral plate pattern of epitheca is "meta" and the middorsal intercalary 2a is quadrandangular or rarely pentagonal. The postcingular row of plate is very narrow, its width being nearly comparable with that of the girdle, and the antapicals are of extremely large plates, forming by far the greater portion of the epitheca. The girdle forms a distinctly ascending spiral, displaced distally about a height of the distal postcingular plate. The ventral area is relatively short, not extending to the center of the epitheca and slightly broadening posteriorly. Its narrower anterior part lies obliquely, while its broader posterior half extends straight in meridional direction. The short antapical spines stand just outside the posterior plate and the inter-antapical suture extends dorsally from the posterior median point of the ventral area. The left side list of the ventral area is restricted basally, in most cases, only along the left margin of the left plate so as to leave the postmargin of the posterior plate free from the list-formation, and the left antapical spine is not connected with the list in any way. The right side list of the area is narrow and indistinct. The longitudinal furrow s. str. is restricted within the left half of the area, not expanding into the right plate.

This group is distinguished from Humilia by its distinct girdle displacement, the anterior oblique extension of the ventral area, the posterior expansion of the ventral area and by the subterminal ending of the left side list, which has no connection to the left antapical spine. *P. roseum* PAULSEN and its allied species seems to be closely related to this group in the structural relation of their ventral area and the distinctly ascending girdle, but differ in their removed right antapical spine, which suggests some closer relations with the preceding group. The monacantha group is the other one to be distinguished from this by its removed single antapical spine when the *roseum*-group were separated from it by further minute examination of their skeletal morphology. The globula group, which is also closely related in some other points, is distinguished from this by its smaller posterior plate, distinctly ascending and overhanging

girdle, and the absence of the antapical spine. Among the subgenus *Protoperidinium*, the group Pellucida has the most highly organized wing-system, guarding the ventral area, and this is the main feature that distinguishes it from the group Humilia.

This group includes following species as valid.

*P. cerasus* PAULSEN (LEBOUR 1925, not PAULSEN's species).

*P. ovatum* (POUCHET) SCHÜTT (PAULSEN 1908, p. 44, Fig. 54; DANGEARD 1927, p. 4, Fig. 3; PETERS 1928, p. 40-41, Fig. 10 a-b).

*P. quanerense* (STEIN) SCHRÖDER (BERGH 1910, p. 184, Fig. 3<sub>I</sub>; BROCH 1910, p. 184, Fig. 3<sub>I</sub>, not II and III; PAULSEN 1931, p. 31, Fig. 32<sub>B-D</sub>).

*P. roseum* PAULSEN (MARUKAWA 1921 b, Fig. 81, Pl. 8, not PAULSEN's original species).

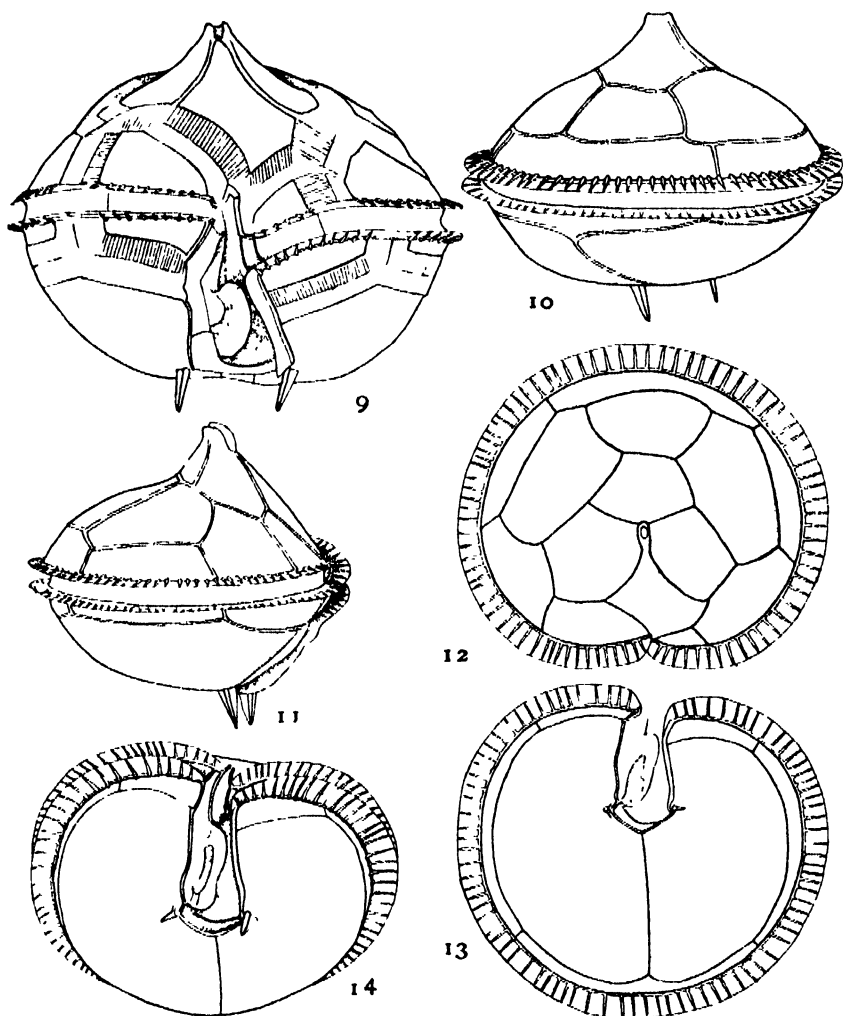
*P. ovatum* LEBOUR (1925) is closely related with *P. lenticulatum* F. F. and may be distinguished from PAULSEN's or DANGEARD's species by their nearly circular girdle.

To this group may be added *P. decipiens* JÖRGENSEN if PAULSEN's Fig. 63 b be accurately drawn. This suggestion is based upon its distinct displacement of the girdle and the typical relation between the proximal end of the girdle and the distal postcingular plate, associated with the flattening of the body.

## 2. *P. marukawai*, n. sp. (Figs. 9-16)

*P. roseum* PAULSEN (MARUKAWA 1921 b, not PAULSEN's).

The body of this species is slightly flattened downwards from apex to antapex in cake- or lense-shaped and its apex and antapical spines are displaced ventrally. But the displacement of the apex of smaller specimen is not so much as that of the larger one. The body of full grown specimen may be more rounded than that of the smaller narrow sutured one, probably due to the strong development of the sutures in the epitheca, which rises above the thecal surface (Fig. 7). But the flattening of the narrow sutured specimen is also variable in some extent. The transverse section of the body at the girdle takes a broad reniform due to a ventral median indentation, and the largest transverse diameter lies in its ventral half in connection with the ventral displacement of the apex and also of the antapex where the antapical spines stand (Figs. 12, 13). The slightly excavated girdle forms a ascending spiral, displaced distally 1-2 girdle



*P. marukawai*, n. sp.

Fig. 9. Ventral view of a wide sutured specimen showing bulged dorsal portion of epitheca caused by growth, ventral area and antapical spines with lateral fin ( $\times 600$ ).

Fig. 10. Dorsal view of another narrow sutured specimen showing normal outline of body and asymmetrical antapical spines ( $\times 600$ ).

Fig. 11. Side view of the same specimen with ventral displacement of apex and antapical spines. Both right and left side lists of ventral area are also illustrated. ( $\times 600$ ).

Fig. 12. Apical view of epitheca ( $\times 600$ ).

Fig. 13. Antapical view of hypotheca showing plate pattern, distribution of ribs in the cingular list and relation of left side list to left antapical spine ( $\times 600$ ).

Fig. 14. Postero-ventral view of hypotheca of another specimen showing typical ventral area ( $\times 600$ ).

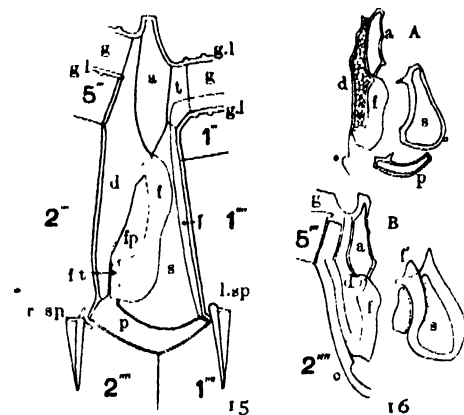
width, which corresponds to the height of the distal postcingular plate, and is guarded with well ribbed lists. The median margin of the distal postcingular plate, accordingly, lies opposite to the proximal end of the girdle, with the anterior part of the ventral area between them. The short ventral area is somewhat oblique in its anterior part, while its posterior broader half lies straight, not extending to the center of the hypotheca (Fig. 9). Two slender spines stand at outside the postero-lateral corners of the ventral area, each with three side fins in most cases, but not rarely one or two of them are extremely narrow or wholly absent.

The ventral plate pattern of epitheca is "meta" and the middorsal intercalary plate 2a, which is trapeziformed or somewhat rounded, is quadrangular and lies either just in the median or slightly displaced to one side, right or left. The slouch in the left half of the ventral apical plate as that of *P. subcurvipes*, is also distinct in this species. Surrounded with two larger lateral and one smaller middle intercalary plates, the dorsal and lateral outer contour of the apical series of plate assumes a trapeziform in apical view (Fig. 12). The dorsal precingular 4'' is a large, transversely elongated plate and the proximal triangular 1'' is the smallest in the series, whose median margin is oblique in accordance with the oblique extension of the anterior plate. The postcingular row consists of extremely narrow plates, and its subterminal components of younger specimen attain in their height only 0.3 1 width of the cingular plate, while the other three have a little larger breadth. The antapicals are extremely large, forming by far the greater portion of the hypotheca. The inter-antapical suture runs straight in dorso-ventral direction, coming in contact with the posterior median point of the ventral area (Fig. 13).

The girdle consists of three plates, whose terminal components correspond in their length with the basal length of the corresponding terminal precingular plate. The ventral area grows broader posteriorly until it ends abruptly in a shallow V-form (Fig. 13). The anterior extension of the small and elongated anterior plate slightly indents the epitheca. The slender right plate extends anteriorly to the epitheca and bears a broad flagellar fin along its posterior median margin. The left plate expands posteriorly, forcing the flagellar pore to take an oblique position in the right half of the ventral area (Fig. 15). Consequently, the minute flagellar trough lies at the posterior right corner of the area in the vicinity of the right antapical spine. The actual length of the trough lying outside the pore can be surmised plainly by a truncated postero-



median margin of the isolated left plate (Fig. 16, A). In old well grown specimen, there may be a very narrow secondary list, springing from the



*P. marukawai*, n. sp.

FIG. 15. Schematized ventral area. a—anterior plate, s—left plate, d—right plate, p—posterior plate, t—transitional plate, l—left side list, f—flagellar fin, fp.—flagellar pore, f.t.—flagellar trough, l.sp.—left antapical spine, r.sp.—right antapical spine, 1''', 5'''—postcingular plates, 1''', 2'''—antapical plates, g.—girdle, g.l.—cingular list.

FIG. 16. Dissociated components of ventral area ( $\times 600$ ) of narrow sutured specimen (A) and broad sutured specimen (B). f'—secondary flagellar fin standing along left margin of flagellar pore. Small rings indicate position of spine.

these spines extend in side view parallel to the body axis or inclines to ventral, not diverging distally. The longitudinal furrow s. str. is restricted in the left half of the area, represented by the anterior, left and posterior plates. The posterior plate forms a minute transverse faint groove, lying along the posterior margin of the ventral area in direct communication with the flagellar trough, and a ridge or a extremely narrow list marks it from the left plate (Fig. 14). The obliquely extending flagellar fin stands not only along the right edge of the flagellar pore but also of the flagellar trough, and in many cases, it extends further anteriorly to the height corresponding to the proximal end of the posterior cingular list. The left side list extends posteriorly to the posterior end of the left plate.

left edge of the pore and lying beneath the primary flagellar fin (Fig. 16 f'). The slightly curved and narrow posterior plate lies transversely at the postmargin of the ventral area. The short antapical spines are of subequal length, standing just outside the posterior plate and rising from the ventral marginal part of the antapical plates. They bear respectively two or three side fins, which are wholly absent in some or only poorly developed in others. In most cases, the left spine has a narrow side fin and sometimes a additional smaller one upon its right inner side. And the latter fin is not related in any way with the left side list of the ventral area. The right spine has three fins, radiating from the shaft of the spine, each in ventral, lateral and median direction. And

Together with the longitudinal furrow s. str., these two fins form a sheath-like channel to accommodate the basal part of the flagella. The right side list is indistinct and interrupted at the postcingular-antapical suture or sometimes restricted only in its anterior portion standing along the median margin of the distal postcingular plate.

The thecal wall is covered with fine meshes with or without minute spines at the nodes. And this texture is also found in the elongated right plate while the other three components are free from the marking (Fig. 16 A).

Dimensions: Body length 53–72  $\mu$ , transverse diameter 56–85  $\mu$ , dorso-ventral diameter 52–75  $\mu$ , width of girdle 4.5–6  $\mu$ .

This species is most closely related to *P. ovatum* (POUCHET) SCHÜTT. The body contour of present species seems to be related to that of those species figured by GRAN (1902), PAULSEN (1908, p. 44, Fig. 54), LEBOUR (1925) DANGEARD (1927) and PETERS (1928). And this seems to be true also concerning the structural relations of their ventral area. DANGEARD's species differs from mine in its small and abruptly differentiated apical horn and more distinct dorso-ventral flattening of the body. But all these PAULSEN's, LEBOUR's and also DANGEARD's species are distinguished from present species by their plate pattern not only of epitheca but also of hypotheca, with a exception of DANGEARD's Fig. 3 d, which shows some closer resemblance with my species. The plate pattern of epitheca of *P. lenticulatum* F. F. (1908, p. 217, Figs. 4, 5, Pl. 15) is nearly the same with that of my species. And I am in different opinion to PAULSEN (1911) who identified this with *P. ovatum*. But above all, our species is distinguished from all these species mentioned above by its very narrow postcingular row of plates.

It seems to me most probable that *P. roseum* illustrated by MARUKAWA et YONEDA (MARUKAWA 1921 b, Fig. 81 c–g, Pl. 8), to which this is most closely related, can be distinguished from my species by its rotund body and removed antapical spines. Strictly speaking, he might have confused two different species because of the fact that Fig. 81 c–d (ventral view) has somewhat removed spines while Fig. 81 g suggests the same structural relation with my species concerning the spine and the ventral area, or if not so, he might have figured incorrectly either of them.

*P. granii* OSTENFELD (PETERS, but not those of PAULSEN and others) is the other one to be considered here, to which my species is closely similar in body form and plate pattern of epitheca, but is to be distinguished by its widely excavated ventral area and also widely divergent

antapical spines standing upon the top of low horn-like protuberances.

### 3. *P. ventralis*, n. sp.

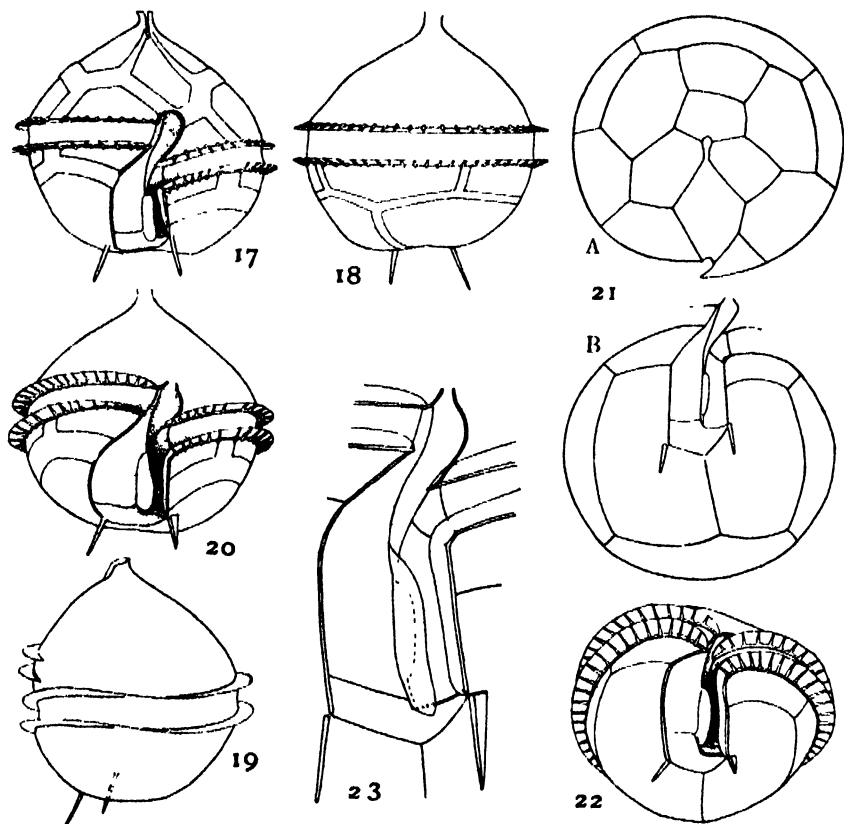
(Figs. 17-23)

This pear-shaped species is characterized by its small size, distinct ascending girdle and two delicate antapical spines. The minute apical horn is distinctly differentiated and flares a little at the aperture. The hemispherical hypotheca has slight antapical flattening.

The ventral plate pattern of epitheca is "meta" and the middorsal intercalary 2a is quadrangular, and the other two are in nearly bilateral balanced position. The postcingular row of plate is narrow and the antapicals are very large, but not so much as that of the preceding species. The left 1''' is smaller than the right 2'''. The interrelations between the minute precingular 1'', the postcingular 5'', the girdle, the interantapical suture and the ventral area are the same, in the main, with those of the preceding species. The girdle forms a complete ascending spiral, displaced distally 1.5 girdle width.

The ventral area is also similar in its structural relation to that of the preceding. It is short, not extending to the center of the hypotheca. The median margins of both the first precingular and the distal postcingular are very oblique in connection with the slantwise extension of the anterior narrower part of the ventral area, and the posterior broader half of the area extends straight in meridional direction. The left side list of the ventral area is narrow and restricted only along the left margin of the left plate. The flagellar fin is narrow and small, standing along the posterior left margin of the right plate and extending anteriorly to the height corresponding to the proximal end of the posterior girdle list. There is another list, very narrow and indistinct, standing along the right margin of the ventral area, which is interrupted at the postcingular-antapical suture (Figs. 17, 20). The flagellar pore lies in the left posterior part of the area in consequence of striking development of the right plate. The slender anterior plate indents the epitheca with its narrow anterior extension. And the small and narrow left plate lies posterior to the minute pentagonal transitional plate, which expands markedly into the ventral area. The large right plate tapers anteriorly to a narrow extension which extends to the epitheca. This unbalanced posterior broadening of the right plate naturally displaces the flagellar pore to the left. The posterior plate consists of broader right and narrower left portions, whose

pointed left end lies at the base of the left antapical spine. The right antapical spine stands outside the right posterior corner of the posterior plate (Fig. 22). These two spines extend ventro-posteriorly and diverge distally. And they are generally wingless, but rarely the left has a narrow and indistinct lateral fin, and is not connected in any way with the left side list of the ventral area. The longitudinal furrow s. str. is



*P. ventralis*, n. sp.

- Fig. 17. Ventral view of a specimen with narrow ventral area ( $\times 600$ ).  
 Fig. 18. Dorsal view of another specimen ( $\times 600$ ).  
 Fig. 19. Side view of the same ( $\times 600$ ).  
 Fig. 20. Ventral view of a different wide stured specimen with extremely broad ventral area ( $\times 600$ ).  
 Fig. 21. Schematized plate patterns of epitheca (A) and of hypotheca (B).  
 Fig. 22. Ventro-antapical view of hypotheca of the first specimen ( $\times 600$ ).  
 Fig. 23. Schematized ventral area.

restricted in the left narrow region, occupied by the left plate and a part of the posterior and anterior plates (Figs. 17, 20).

Dimensions: Body length 38-41  $\mu$ , transverse diameter 40-43  $\mu$ , dorso-ventral diameter 37-40  $\mu$ , width of girdle 5-6  $\mu$ .

This species is very closely related to *P. quarnerense* (BROCH, 1910, p. 184, Fig. 3<sub>I</sub>, not <sub>II</sub>; DANGEARD 1927, Fig. 9; PAULSEN 1931, Fig. 32). But these illustrated by them are distinguished from mine by their more swollen body and abruptly differentiated apical horn. It seems to me probable that *P. cerasus* illustrated by most of the former investigators do not represent a one and the same species in every cases. And among them BROCH's species is most closely related to this in its plate pattern of epitheca and also in the outline of the ventral area. From *P. cerasus* PAULSEN (1908, p. 52; PETERS 1908, Fig. 12 a-d) and *P. roseum* PAULSEN (1908, p. 44, Fig. 53) are distinguished from this by their removed ant-apical spines. From *P. marukawai* to which this is most closely related, it is to be distinguished by its body form, smaller size, pronounced oblique extension of the anterior part of the ventral area, and distinctly expanded posterior part of the area coupled with its characteristic posterior plate. Judging from SABELINA's figure 4 a, c, *P. turgidum* MEUNIER (SABELINA 1930, Fig. 4) differs from this in the plate pattern of epitheca and relative size of the terminal postcingular plates, though much resembles each other in body form. And *P. sylvanae* DANG. (1932, fig. IV, a-f) seems to differ from this not only in body form and cingular displacement but also in the organization of the hypothecal appendages. Detailed description or figures of all these species mentioned above have not been given in reference to their skeletal morphology especially of their ventral area. And this makes the identification very difficult. Accepting all these published figures correct and yet I am inclined to regard my species a different one.

### III. GROUP PYRIFORMIA

JÖRGENSEN grouped *P. steini* and its allied species under his fourth section Pyriformia. And recently PAULSEN (1931), following JÖRGENSEN's system, placed it in his fifth section and cited seventeen species as valid. And present group intended to define in this paper, includes a part of PAULSEN's section with following modifications.

The globular or pear-shaped body has a circular and rarely oval cross section. The apical horn exists in most cases. The girdle is circular or

slightly ascending and is guarded with broad hyaline or well ribbed lists. The hemispherical hypotheca has two antapical spines at the posterior lateral margin of the ventral area.

This group is characterized especially in the structural relations of the ventral areas and its appendages. The ventral area is straight, extending in meridional direction and slightly indenting the epitheca. It is widest at the postcingular-antapical suture and narrows posteriorly, terminating, in most cases, in bluntly pointed end. The left side list stands along the whole length of the left margin of the left plate. The left antapical spine stands at the left anterior end of the posterior plate, springing from the median margin of the left antapical plate. It is connected, in most cases, directly with the left side list, but rarely it extends independently outside the list without any direct connection. Thus the left side list is divided, in most cases, by the spine into two portions, the one forming the ventral and the other the dorsal wings of the spine. And its ventral wing is connected with the posterior cingular-list through the left side list of the ventral area, and the posterior fin stands along the sinistro-posterior margin of the posterior plate, ending at the ventral end of the interantapical suture. But rarely these two fins, as stated above, form a continuous wing without any direct connection with the spine. In highly organized species, there is a minute third wing, the side fin of the spine. The right antapical spine stands either immediately outside the right posterior corner of the posterior plate or at a little distance from there on the right antapical plate. The right spine is wingless or buttressed with one or two wings, and in most highly organized species there are three wings radiating from the shaft of the spine respectively in ventral, dorso-median and lateral directions. Generally, they are small and indistinct, but in most highly organized species the ventral one extends anteriorly coming in connection with the distal end of the posterior girdle list in the same way as that of the left spine. The right side list of the ventral area is variable in different species, but it is generally narrow or indistinct as compared with the highly developed left one. There is a narrow fin, in most cases, standing along the median margin of the postcingular plate 5'', which, in some highly organized ones, is discontinuous with the ventral fin of the right antapical spine. The large flagellar fin stands along the right edge of the flagellar pore with a minute spine in its anterior end. But not rarely, it extends further anteriorly beyond this spine, for a short distance, along the suture between the anterior and the right plates. The flagellar fin of this group is larger and more distinct as compared with that of the pellucida group,

whose side list is the most highly developed and specialized one among the genus *Peridinium*.

The ventral plate pattern of the epitheca is "meta", and the mid-dorsal intercalary is pentagonal or rarely quadrangular. The three anterior intercalary plates are slightly displaced, as a whole, to the left. The postcingular row of plates is not narrow, and its two terminal plates, I''' and 5''', extend posteriorly nearly to or further than the midway between the girdle and the posterior end of the ventral area. The length of the terminal cingular plates corresponds to the basal length of the corresponding terminal pre- or postcingular plates. The transitional plate is small and narrow.

Among the four components of the ventral area, the largest right plate extends anteriorly to the epitheca and narrows posteriorly, terminating in more or less pointed end. The left plate, on the contrary, broadens posteriorly and its postmargin does not bear a distinct lip. Connected with the asymmetry in the posterior halves of these median two plates, the flagellar pore lies obliquely. The posterior plate is small, and its transverse diameter is much smaller than that of the median part of the ventral area. This plate can be divided into a narrow and longer left, and a wide and shorter right portions, and the inter-antapical suture extends posteriorly, in most cases, from the median point of its postmargin. Rarely, the posterior plate is quadrangular with the inter-antapical suture springing from its left posterior corner. This latter type of the posterior plate seems to designate a transitional form between this and the pellucida group.

Two forms can be detected in the texture of the thecal wall. The thecal wall of *P. steini* is covered with sparsely scattered minute pores, while that of *P. subpyriforme* with fine meshes. In the latter case, the right plate, which is the sole protuberant one among the quadruplet, has the same texture with that of the body plate, and the other three are free from the marking. This seems to be related with differentiation of the longitudinal furrow s. str.

The Humilia and the Pellucida groups are the most closely related ones to this. But present group is distinguished from the former by its circular girdle, smaller posterior plate, the asymmetrical development of the posterior part of the middle two plates of the ventral area, and the direct connection of the left antapical spine with the left side list of the ventral area or the further posterior extension of the list. From the Pellucida group, this is also distinguished by its smaller posterior plate, absence of

a minute wing connecting the side list of the ventral area with the left antapical spine, absence of a minute pore at the anterior median corner of the first postcingular plate, which we found in all the species of the group and shall be described in a later paper.

From my investigation on the ventral area of some of this group and a critical review of literatures and published figures, following species may be recognized to be included in this group.

*Peridinium heteracanthum* DANGEARD (1927, p. 7, Fig. 35).

*P. longicollum* PAVILARD (PAULSEN 1931, p. 63, Fig. 35).

*P. michaelis* EHBG. (SCHÜTT 1895, Pl. 14, Fig. 16).

*P. oviforme* DANGEAD (1927, p. 4, Fig. 2; PAULSEN 1931, p. 62, Fig. 34; DANG. 1932, Fig. 4 a-c).

*P. steini* JÖRGENSEN (PAULSEN 1908, p. 47, Fig. 58; KOFOID 1909, Figs. 1-7, Pl. 2; BROCH 1910, p. 185, Fig. 1; DANG. 1932, Fig. V c; LEBOUR 1925, Pl. 14, Fig. 4 a-d).

*P. steini* v. *africanum* DANG. (1927, p. 2, Fig. 1<sub>D-F</sub>).

*P. sylvanae* DANG. (1927, p. 2, Fig. 1<sub>A-C</sub>).

*P. variegatum* PETERS (1928, Fig. 9 a-g).

Here may be placed, also, following species, but as their morphological details are insufficiently known at present, careful examination of their thecal structure is necessary before precise decision is made as to their actual position.

*Peridinium pedunculatum* SCHÜTT (PAULSEN 1908, p. 47, Fig. 59).

*P. latisspinum* MANGIN (1922, p. 81, Fig. 24<sub>1</sub>).

*P. longispinum* KOFOID (1907, Fig. 33, Pl. 5).

*P. castaneiforme* MANGIN (1922, p. 79, Fig. 20<sub>2</sub>).

*P. breve* PAULSEN (1908, p. 46, Figs. 5-6; 1911, p. 309, Fig. 7, non LEBOUR 1925).

*P. rectum* KOFOID (1907, p. 311, Figs. 48-49).

*P. subpyriforme* DANG. (1932, Fig. V a-b).

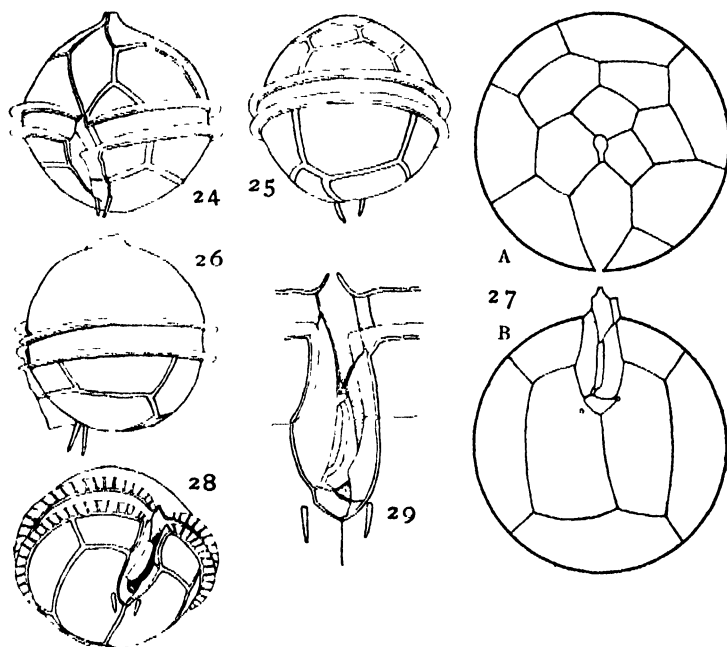
#### 4. *P. solitarium*, n. sp. (Figs. 24-29)

The body of this minute species is globular with a minute but abruptly differentiated apical horn and somewhat ventrally displaced short antapical spines. The slightly ascending median girdle is guarded with well ribbed lists.

The plate pattern of epitheca is typical for the group Pyriformia.



The ventral plate pattern is "meta" and the ventral apical plate is somewhat elongated pentagonal and the first precingular a small triangular plate. Three anterior intercalaries are displaced as a whole to the left. In consequence of this asymmetry, the left apical is smaller than the right. The postcingular plates are arranged as to increase their height successively from ventral to dorsal, and the middorsal 3''' is the tallest and



*P. solitarium*, n. sp.

Fig. 24. Oblique ventral view ( $\times 600$ ).

Fig. 25. Postero-dorsal view ( $\times 600$ ).

Fig. 26. Side view showing antapical spine and left side list of ventral area ( $\times 600$ ).

Fig. 27. Schematized plate patterns of epitheca (A) and of hypotheca (B).

Fig. 28. Oblique antapical view of hypotheca showing well ribbed ringular lists and relation between antapical spine and side list of ventral area ( $\times 600$ )

Fig. 29. Surface view of ventral area ( $\times 1500$ ).

largest plate among them, extending posteriorly occupying more than a quarter of the dorso-ventral surface length of the hypotheca. The oblique inter-antapical suture separates the smaller left antapical plate from the larger right (Figs. 25-27).

The girdle forms a slightly ascending spiral with its distal displacement by 0.3 its width. The exceedingly narrow transional plate lies transversely at the proximal end of the girdle.

The ventral area is short and narrow, not extending to the center of the hypotheca. It broadens a little at the postcingular-antapical suture and then narrows posteriorly, terminating in bluntly pointed end. The hyaline left side list broadens posteriorly and forms a wide marginal serra before it terminates (Fig. 26). This is not connected in any way with the left antapical spine and bears a short rib at the postcingular-antapical suture. The anterior plate slightly indents the epitheca. Concerning the subequal median plates, the left component slightly broadens posteriorly, while the irregularly spindle-shaped right plate extends anteriorly to the midway between the two distal ends of the girdle lists. The flagellar pore lies slightly oblique between them. The minute posterior plate is bluntly pointed posteriorly. The flagellar fin has a minute subterminal rib at the anterior end of the flagellar pore. The ventral area is slightly depressed as a whole, but its left half forms especially a deeper groove or the longitudinal furrow s. str. (Figs. 28, 29).

The short antapical spines are wingless, and the left one inclines and locates more ventrally than the right, standing just outside the left pointed end of the posterior plate, while the right stands at a distance from the posterior plate, springing from the right antapical plate (Fig. 29).

Dimension: Body length  $36\ \mu$ , transverse diameter  $36\ \mu$ , dorso-ventral diameter  $35\ \mu$ , breadth of the girdle  $5.4\text{--}6\ \mu$ , width of girdle  $2\text{--}2.5\ \mu$ .

This species is distinguished from *P. subpyriforme* DANG. to which it is most closely related, by its smaller size, larger dorsal postcingular plate  $2'''$  and isolated left antapical spine. *P. truncus* n. sp. is also closely related to this in size and outline of the body, but is distinguished by its larger ventral area. *P. ventralis* n. sp. is distinguished from this by its narrow postcingular row of plates, more strongly ascending girdle, and oblique elongation of anterior part of the ventral area. From *P. nipponicum* ABÉ it is distinguished by its simpler organization of the ventral area. And from *P. cerasus* it is distinguished at once by its smaller antapical horn and more closely lying antapical spines.

5. *P. subpyriforme* DANGEARD

(Figs. 30-37)

DANGEARD 1932.

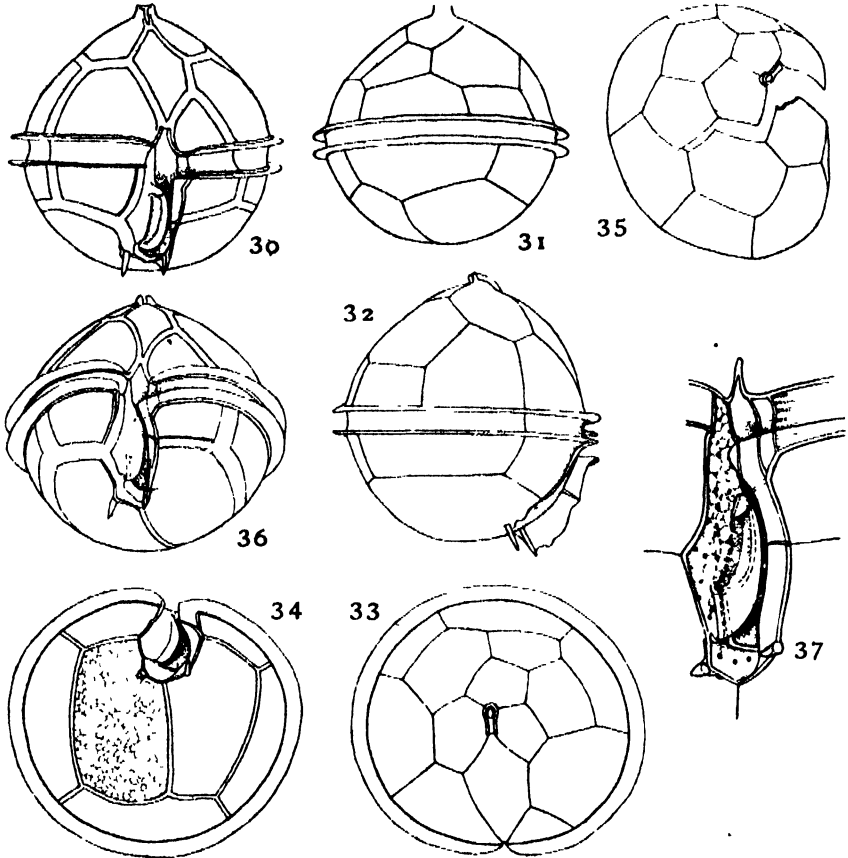
This is a medium sized globular species with a minute apical horn and ventrally displaced short antapical spines. The swollen conical epitheca is slightly larger than the hemispherical hypotheca, and the slightly ascending postmedian girdle has faintly depressed wall and is guarded with hyaline lists.

The plate pattern of epitheca is typical for the group *Pyriformia* in most cases. The ventral plate pattern of epitheca is "meta" and the ventral apical is elongated pentagonal. The first precingular plate is much smaller than the distal. Among the three intercalaries, the pentagonal middle plate is the smallest, and displaced to the left wedging in between the precingular 3'' and 4''. But rarely I found specimens with quadrangular intercalary 2 a. The postcingular series is narrow, its breadth not exceeding half the height of the hypotheca, and the proximal 1''' is smaller than the distal 5'''. The antapicals are subequal, the left being smaller than the right in consequence of the oblique extension of the inter-antapical suture (Figs. 33, 34).

The girdle consists of three plates and its terminal two correspond in length with the basal length of the terminal plates of the pre- or postcingular row. The extremely narrow transitional plate lies transversely at the proximal end of the girdle (Fig. 30).

The small ventral area is short and narrow, not extending to the center of the hypotheca. It is widest at the posterior-antapical suture and again narrows posteriorly. The small anterior plate slightly indents the epitheca with its anterior narrow extension. The irregularly elongated right plate extends anteriorly to the epitheca, and posteriorly narrows to a pointed end. The small J-shaped left plate extends anteriorly to the proximal end of the posterior cingular list, and broadens posteriorly into a rectangular part, which bears along its median margin the narrow and elongated flagellar trough. The flagellar pore is relatively short, lying somewhat obliquely in the center of the area. The trough lies in the right of the area. The irregular minute posterior plate is pointed to the left, terminating at the left antapical spine. The wingless right antapical spine stands at the right posterior corner of the posterior plate, rising from the median margin of the right antapical plate. This is especially apparent in broad sutured specimens (Figs. 36, 37). In specimens such as illustrated

in Fig. 30 or 36, there is broad band or zone of growth lying along the right outer margin of the ventral area, separating the areal plates from the other body ones, and consequently the right antapical spine seems



*P. subpyriforme* DANGEARD

Fig. 30. Ventral view ( $\times 600$ ).

Fig. 31. Dorsal view of another smaller specimen ( $\times 600$ ).

Fig. 32. A side view of the larger specimen showing antapical spine and side lists of ventral area ( $\times 600$ ).

Fig. 33. Apical view of epitheca ( $\times 600$ ).

Fig. 34. Antapical view of hypotheca showing side list of ventral area and marking of thecal wall ( $\times 600$ ).

Fig. 35. Oblique apical view of partially dissociated epitheca showing apical closing platelet ( $\times 600$ ).

Fig. 36. Postero-ventral view of a different specimen ( $\times 600$ ).

Fig. 37. Surface view of ventral area ( $\times 1500$ ).

to be removed from the posterior plate. The left antapical spine, provided with ventral and ventro-median wings, stands at the left pointed end of the posterior plate, rising also from the median margin of the left antapical plate. The narrow and triangular posterior fin of this spine stands along the sinistro-posterior margin of the posterior plate, terminating at the ventral end of the inter-antapical suture. Its broad ventral fin is connected with the meridional extension of the posterior girdle list at a rib at the postcingular-antapical suture, thus forming the left side list of the ventral area (Fig. 37). The broad flagellar fin seems to guard both the flagellar pore and the flagellar trough, and bears a minute nail-like spine or process in its anterior end at the anterior end of the flagellar pore. The distal free margins of these flagellar fin and right side list run parallel for the most part, thus canopying the most part of the longitudinal fulrow s. sir. which extends further anteriorly and also posteriorly leaving only the right plate outside the canal thus formed.

It may be worth to note here that I have often observed specimens with three distinct short ridges, radiating from the base of the right antapical spine, each in ventral, lateral and postmedian direction (Figs. 30, 37). These ridges may correspond apparently in their position and direction to those of the three wings of the corresponding spine of *P. steini*.

The thecal wall is covered with fine meshes and crowded pores at the nodes. In the ventral area, the right plate has the same marking, while there are only three or four minute pores in the posterior plate. And the other two are hyaline without any marking or porulation (Fig. 37). This may be due to their structural and also functional differentiation.

Dimensions: Body length 40-50  $\mu$ , transverse diameter 43-52  $\mu$ , dorso-ventral diameter 36-45  $\mu$ , width of girdle 4-5  $\mu$ .

By the slender form of the midventral apical plate, present species seems to be distinguished, if any, from DANGEARD's species (1932, Fig. Va-b). And another point is its swollen epitheca. But, disregarding these points, both these species seem to me to be identical. *P. variegatum* PETERES (1928) is also closely related to this, except its ventral plate pattern (para) of epitheca.

#### 6. *P. truncus*, n. sp.

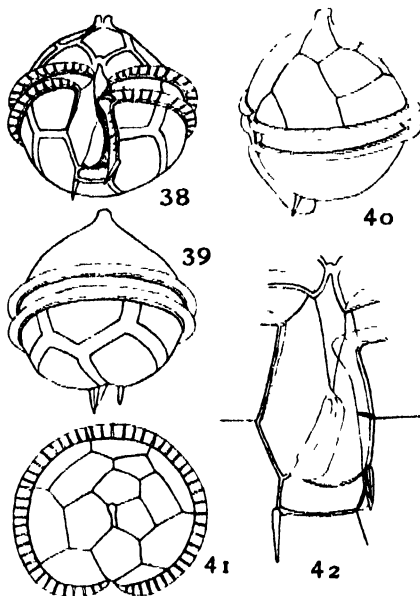
(Figs. 38-42)

This minute species is characterized by its trochoidal body and relatively large, posteriorly truncated ventral area. The transverse section of the

body is nearly circular with faint ventral flattening. The girdle forms a slightly ascending spiral, displaced distally 0.5 its width and is guarded with well ribbed lists.

The plate pattern of epitheca is typical for the group. The postcingular row is relatively wide and the left antapical plate is smaller than the right.

The relatively large ventral area extends nearly to the center of the hypotheca. It is widest, as in common in the group, at the postcingular-antapical suture and again slightly narrows posteriorly. The minute anterior plate indents the epitheca anteriorly and tapers posteriorly to a pointed end. The left plate extends anteriorly to the midway between the two proximal ends of the cingular edges, and broadens posteriorly. The elongated large pentagonal right plate with bluntly pointed ends, extends anteriorly to the epitheca. And the flagellar pore lies slantwise in the middle of the posterior part of the ventral area and has the very short flagellar trough. The posterior plate is similar to that of the previous species but a little larger, and the ventral end of the inter-antapical suture is displaced to its left posterior corner. The short left antapical spine stands at the left anterior corner of the posterior plate, extending ventro-posteriorly and has its ventral and dorsal wings similar to those of the previous species. The wingless right spine stands at the right posterior corner of the same plate, extending posteriorly. The left side list of the ventral area broadens posteriorly, and we can see, in rare cases, a line of several minute ribs along its basal part, in addition to a stout rib at the postcingular-antapical suture. The right side list is



*P. truncus*, n. sp.

Fig. 38. Postero-ventral view ( $\times 600$ ).

Fig. 39. Oblique dorsal view ( $\times 600$ ).

Fig. 40. Oblique side view ( $\times 600$ ).

Fig. 41. Apical view of epitheca ( $\times 600$ ).

Fig. 42. Ventral view of half schematized ventral area ( $\times 1500$ ).

very narrow and restricted only in its anterior part along the median margin of the distal postcingular plate. The small flagellar fin is similar to that of the previous species in its relation to the flagellar pore and the short flagellar trough. The longitudinal furrow s. str. is restricted, as usual, in the left half of the ventral area excluding the right plate (Figs. 38, 42).

Dimensions: Body length  $24\ \mu$ , transverse diameter  $31\ \mu$ , dorso-ventral diameter  $28\ \mu$ , width of girdle  $4\ \mu$ .

By its large ventral area and its quadrangular posterior plate, this species is distinguished from *P. steini*, *P. solitarium* and *P. subpyriforme*. And from *P. nipponica* it is distinguished by its simpler fin-and-wing-system around the ventral area. *P. ventralis* is distinguished from this by its narrow postcingular row of plates, its more distinct ascending girdle and oblique anterior extension of its ventral area.

## 7. *P. pyriforme* PAULSEN

(Figs 43-50)

PAULSEN 1908, Fig. 57.

„ 1911, Fig. 8.

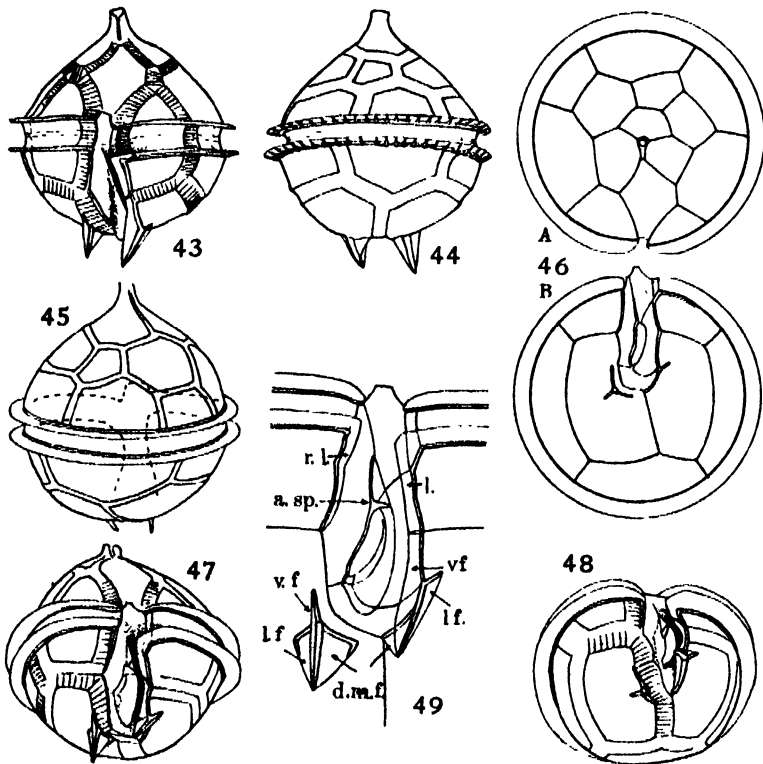
PETERS 1928, Fig. 14.

The spheroidal body of this species tapers anteriorly to a short apical horn and has two short and winged antapical spines. The relatively wide girdle forms a slightly ascending spiral and is guarded with well ribbed list. The ventral area is long, extending posteriorly to the center of the hypotheca.

The plate pattern of epitheca is typical for the group Pyriformia and corresponds with that described by BROCH (1910) for *P. steini*.

The ventral area is relatively wide throughout its whole length, but widest at the postcingular-antapical suture (Fig. 50), and its posterior end is bluntly pointed. The irregularly hexagonal, elongated anterior plate slightly indents the epitheca with its anterior truncated end, and tapers posteriorly. The short J-shaped left plate has rounded broad postmargin and extends anteriorly nearly to the proximal end of the posterior cingular list, terminating at the lower end of the narrow transitional plate, which lies transversely at the proximal end of the girdle. The elongated right plate extends anteriorly to the epitheca, and narrows posteriorly to a truncated end. The flagellar pore is reniform, lying obliquely between the middle plates and the short flagellar trough lies, for the most part, within the flagellar pore. The longitudinal furrow s. str. is restricted in

the region similar to that of the other species of this group. The large flagellar fin extends anteriorly, beyond the rib at the anterior end of the pore, standing along the median margin of the right plate, to the level corresponding to the proximal end of the posterior circular edge. Posterior marginal part of this curved, scoop-shaped flagellar fin turns abruptly to the left to form a narrow folding at the postmargin of the flagellar trough (Fig. 50). This posterior folding is apt to be mistaken for a rib or spine springing from the posterior end of the pore and extending along



*P. pyriforme* PAULSEN

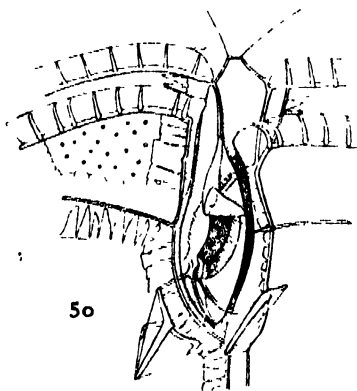
- Fig. 43. Ventral view ( $\times 600$ ).  
 Fig. 44. Dorsal view ( $\times 600$ ).  
 Fig. 45. Dorsal view of another specimen ( $\times 600$ ).  
 Fig. 46. Schematized plate pattern of epitheca (A) and of hypotheca (B).  
 Fig. 47. Postero-ventral view ( $\times 600$ ).  
 Fig. 48. Oblique polar view of hypotheca ( $\times 600$ ).  
 Fig. 49. Schematized ventral area. r.l.—right side list, v.f.—ventral fin, a.sp.—spine at anterior end of flagellar pore, d.m.f.—postero-median fin, l.f.—lateral fin.



the postmargin of the flagellar fin. The small curved posterior plate lies obliquely behind the two median plates, extending between the two ant-apical spines.

The relations of the two antapical spines to the posterior plate are the same with the preceding species. The left spine extends slightly oblique in ventro-posterior direction while the right posteriorly. They are provided respectively with three hyaline fins, which extends radially from the shaft of the spine. The ventral and the dorso-median fins of the right spine are decurrent basally parallel with the dextro-posterior margin of the posterior plate, and the other lateral fin extends in dorso-lateral direction. These three fins are subequal both in size and basal length. The dorso-median one has a terminal minute rib or spine standing at a short distance from the ventral end of the inter-antapical suture. The lateral fin has also a similar spine at its distal end. But these terminal

spines do not always exist but may be absent in some other cases. The ventral fin extends ventrally for about one-third of the distance between the base of the right spine and the median end of the postcingular-antapical suture. The three fins of the left antapical spine are variable in size and grouped also in a similar manner. The lateral fin of the left is the smallest among them, extending in ventro-lateral direction. The broad triangular dorso-median fin is decurrent basally upon the ventro-median margin of the left antapical plate, terminating at the ventral end of the inter-antapical suture. The elongated triangular largest ventral fin extends anteriorly along the antero-median



*P. pyriforme* PAULSEN

Fig. 50. Surface view of ventral area showing, in addition to general features of ventral area itself, well ribbed girdle list, striated intercalary zone and distribution of pores of thecal plate ( $\times 1500$ ).

margin of the same plate, to a rib at the postcingular-antapical suture. Together with the posterior median elongation of the posterior cingular list, this constitutes the left side list of the ventral area. And the rib at the junction is due to their marginal interlocking. The marginal part of the left side list runs parallel with that of the flagellar fin, covering nearly the whole area of the longitudinal furrow s. str. The meridional

posterior elongation of the distal end of the posterior cingular list forms a very narrow right side list, its base terminating at or shortly above the postcingular-antapical suture.

The antapical appendages standing around the ventral area of *P. steini*, and described and elaborately figured by KOFOID, are nearly identical in the main with those of our species. Disregarding the plate pattern of the ventral area, the antapical spine of these two species is provided with three fins grouped in a similar manner, and the right spine is removed, also in both cases, for a short distance from the ventral area. These may be one of the most reliable bases for regarding them very closely related. But the structural relation of the posterior part of their ventral area is wholly different, granting his figures be correctly drawn. The ridge-like structure, which is illustrated in some of his figures, connecting the bases of its two antapical spines and passing around the ventral area, is a character of much higher type such as the group Conica or Pellucida. And it seems to me highly probable that the structure in his figures may be due to his misinterpretation or confusion of more than two species belonging to different groups. This suggestion is based upon my observation which proves the existence of such a structure as his in some of the higher type of the group Pellucida.

The thecal wall is hyaline and covered with corrugated papulation.

Dimensions: Body length, excluding apical horn 38-42  $\mu$ , transverse diameter 37-60  $\mu$ , dorso-ventral diameter 34-38  $\mu$ , width of girdle 5.5-6  $\mu$ .

This pacific minute species seems to be most closely related either to *P. steini* or *P. pyriforme* in some points or others. But from *P. steini*, it is distinguished by its shorter antapical spines, rounded midbody and shorter and apparently differentiated apical horn. *P. pyriforme* seems to be variable in its ratio of body length to breadth, as culculated from PAULSEN's and PETERS's figures, ranging from 0.82 to 0.92, and that of our species is 0.95. Our species is, then, more rotund than the atlantic one, and this is especially apparent in the form of epitheca. PAULSEN's (1911) species may be a different one because of its very narrow ventral apical plate, while our species has a broad one. PETERS's (1928) species seems to me not a single species judging from his figures 14 a-c, because of their variable breadth of the ventral apical plate and of their ventral area. Besides these, his species has a exceedingly large right anterior intercalary plate. The possibility arises, then, that when the minute skeletal morphology of these atlantic species is known, it may be necessary to transfer our pacific species to another or to create a new

species to place present species, but at present lack of adequate minute descriptions of those atlantic species preclude a possible identification. *P. variegatum* PETERS has much shorter spines and more flattened body than our species. *P. michaelis*, *P. oviforme* and *P. longicollum*, also, can be distinguished from this by their elongated midbody and longer antapical spines, while on the other hand, present species can be distinguished from *P. heteracanthum* and *P. subpyriforme* by its elongated body.

**SOME NOTES ON *SPHAERIUM JAPONICUM BIWAENSE*  
MORI<sup>1)</sup>, A FRESHWATER BIVALVE  
IV. GASTRULA AND FETAL LARVA**

By

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(With Plates I-II and ten text figures)

(Received February 27, 1936)

In the development of the species under discussion, the free swimming stage of the veliger is non-existent, the whole embryonic and larval stages being passed in the marsupial sac of the mother (OKADA, 1935 b). In this paper the present writer proposes to describe the gastrulation and the subsequent differentiations, which occur in the larval stage, in general comparison with those in the corresponding stages of Lamellibranchia.

The writer has to express his deep sense of gratitude to Prof. Dr. F. NOMURA for his kind advice given during the course of this investigation.

**MATERIAL AND METHOD**

In his previous papers (OKADA, 1935 a, b and c), the present writer used the name, *Musculium heterodon*, for the species under discussion, because of the presence of the embryonic shell (prodissoconch) attached to its umbo. In the recent taxonomy of Japanese *Sphaerium* as revised by MORI, however, *Musculium heterodon* (PILSBRY) has been adopted as a synonym for *Sphaerium japonicum* (WESTERLUND). According to MORI (1933 and '35), the genus *Musculium*, which has a prominent prodissoconch, had been distinguished from the genus *Sphaerium*, which has an indistinct prodissoconch; but on examining a great number of specimens from the southern Kurile Islands, Hokkaido, Honshyû, Shikoku, Kyûshyû and Quelpart Island, he found that such a distinction is inadmissible, because there is a gradual transition between 'calyculated' and 'non-calyculated' specimens; and the publication of *Calyculina japonicum* WESTERLUND, 1883,

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<sup>1)</sup>*Sphaerium japonicum* is the same species as *Musculium heterodon*. According to S. MORI, the specimens described in the first two of my previous papers (OKADA, 1935 a and b) were identical with *Sphaerium japonicum japonicum* (WESTERLUND), and those in the third (OKADA, 1935 c) with *Sphaerium japonicum biwaense* MORI.

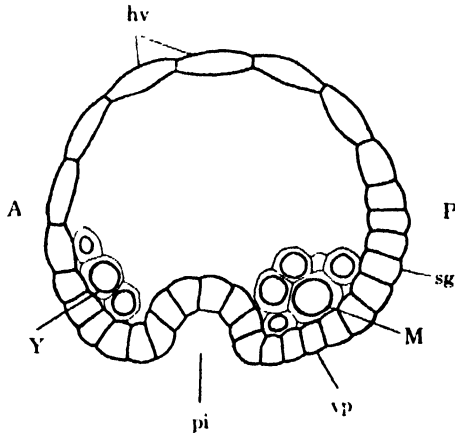
had anticipated the use of the designation *Sphaerium heterodon* PILSBRY, 1895. The present writer is very grateful to Mr. SYUICHI MORI for his kindness in identifying the present specimen with *Sphaerium japonicum biwaense* MORI.

The specimens used in the present study were collected in the autumn of 1934, in a drainage ditch at Iwanuma, a town south of Sendai. Paraffin serial sections of the inner gills of the fixed specimens were prepared 7–10  $\mu$  in thickness. The use of Zenker's solution without glacial acetic acid and of Mallory's connective tissue stain were most favourable in facilitating observation. Moreover, the live embryos, which were removed from the marsupial sac by dissection, were used for the observation of the external characters.

#### OBSERVATION OF THE GASTRULATION

**BEGINNING OF THE GASTRULATION.** At the end of the blastula stage, the cells in the region of the original vegetal pole undergo more frequent divisions than those in the other regions, and the floor becomes thickened

(OKADA, 1935 c). This thickened floor begins to flatten and invaginate into the blastocoel (Text-fig. 1). The outline of this early gastrula is roundish, having a sac-like depression at one end, which is the rudimentary archenteron. The size of the embryo, at this stage, differs considerably with the individual. ZIEGLER (1885) states in the case of *Cyclas cornea* that the diameter of the larger embryos is often half as long again as that of the smaller ones. Such an individual difference in size appears to be caused by some conditions in the limited space in the mar-

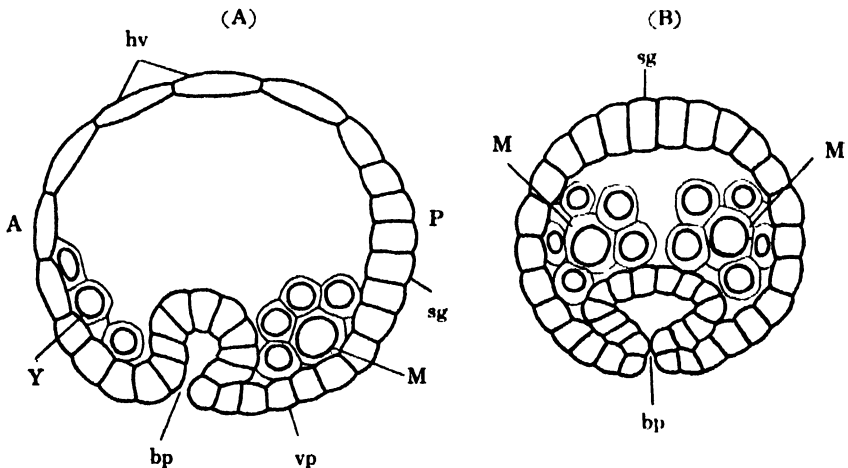


Text-fig. 1. Schematic representation of median, sagittal section of early gastrula.  $\times 400$ . A anterior end of embryo, P posterior end of embryo, hv head-vesicle, M mesoblastic teloblast, pi archenteron, sg cell-plate of rudimentary shell gland, vp ventral plate, Y larval mesoblast.

supial sac, in which a number of embryos are reared in a group. In the species under consideration the normal embryos at this stage have a

diameter of about 80–100  $\mu$ , with the exception of abnormal giant and dwarf embryos.

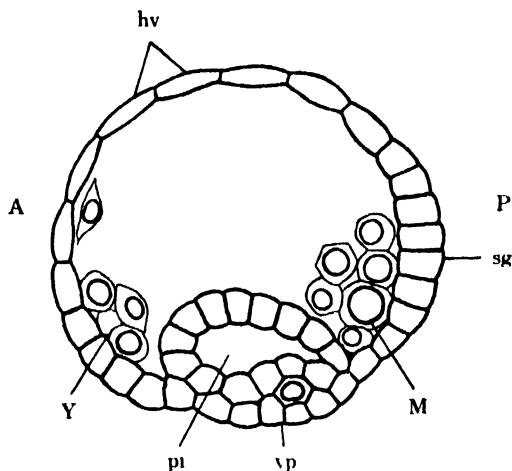
**FORMATION OF THE ARCHENTERON.** The ectoderm at the original animal pole becomes flattened and is composed of large cells (Text-fig. 1 *hv*), each of these having a thin cytoplasm and a flattened large nucleus (Pl.-fig. 1). In the study of *Cyclas cornea*, ZIEGLER (1885) and STAUFFACHER (1894) termed this portion of the ectoderm the 'head-vesicle.' The ectoderm, forming the anterior lip of the blastopore, consists of cubical cells. The ectoderm at the posterior region differentiates into two portions. One consists of original cells of the shell gland, which are the derivatives of larger members X (OKADA, 1935 c, Table 2), the descendants of the first somatoblast, and forms a thickened cell-plate consisting of large columnar elements at the posterior region of the embryo (Text-fig. 1 *sg*). The other consists of original cells of the ventral plate, which are the derivatives of smaller members such as  $2d_2$ ,  $2d_{12}$ ,  $2d_{112}$  and  $2d_{112}$  (OKADA, 1935 c, Table 2), also the descendants of the first somatoblast, and occupies the region from the posterior lip of the blastopore upwards to the thickened cell-plate of the rudimentary shell gland, forming a thinner cell-plate at the postero-ventral side of the embryo (Text-fig. 1 *vp*). In this stage, three or four larval mesoblastic cells are seen forming a cell-mass in the blastocoel near the anterior floor at the original position of Y (Text-fig. 1 *Y*),



Text-fig. 2. Schematic representation of median, sagittal section (A) and of frontal section through blastopore and teloblasts of gastrula in stage of closing blastopore (B).  $\times 400$ . A anterior end of embryo, P posterior end of embryo, bp blastopore, hv head-vesicle, M teloblastic cell-mass, sg cell-plate of rudimentary shell gland, vp ventral plate, Y larval mesoblast.

and the mesoblastic teloblasts, forming bilateral cell-masses, are seen behind the invaginated endoderm, just at the original position of *M* (Text-fig. 1 *M*). Each teloblast consists of a large and several small cells. Further invagination of the endoderm continues and produces the archenteron (Text-fig. 1 *pi*). The process of invagination finishes, seemingly, at the stage when the invaginated roof advances about one third of the diameter of the blastocoel from the floor, and the blastopore soon begins to be closed.

**CLOSURE OF THE BLASTOPORE.** At first posterior and postero-lateral lips of the blastopore begin to grow forward over the blastopore (Text-fig. 2 and Pl.-fig. 2), and then meet and fuse eventually with the anterior



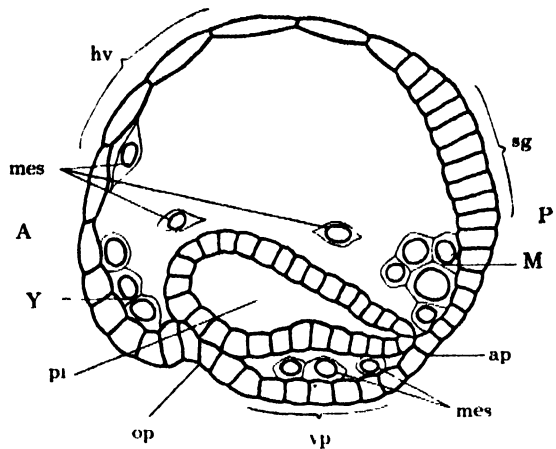
Text-fig. 3. Schematic representation of median, sagittal section of later gastrula having closed blastopore.  $\times 400$ . *A* anterior end of embryo, *P* posterior end of embryo, *hv* head-vesicle, *M* teloblastic cell-mass of mesoderm, *pi* primitive gut, *sg* cell-plate of rudimentary shell gland, *vp* ventral plate, *Y* larval mesoblast

and antero-lateral lips at their original position (Pl.-fig. 3). This forward growth of the posterior and the postero-lateral lips of the blastopore are produced practically by the forward growth of the ventral plate. At the same time, the cell-plate of the rudimentary shell gland begins to move upwards to the dorsal side from its original posterior side of the gastrula, as the effect of the rapid growth of the posterior region. Meanwhile, a slight sign of the ectodermal invagination begins to appear at the original position of the anterior lip of the blastopore (Pl.-fig. 4).

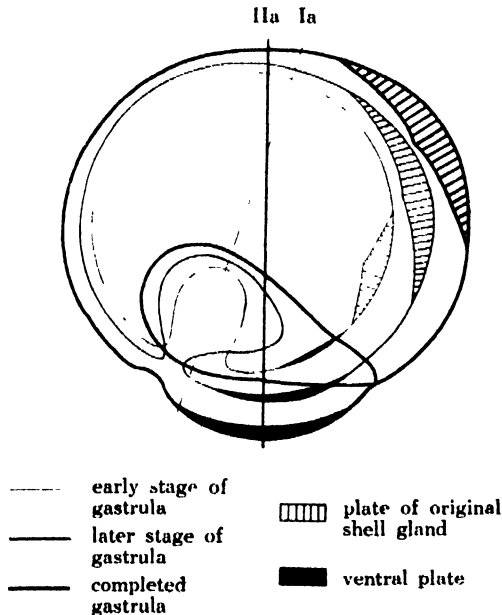
The ectodermal wall of this invagination forms the oral plate together with the anterior wall of the primitive gut. This gut, especially its postero-dorsal portion, extends posteriorly, in the opposite direction to the forward growth of the ventral plate (Text-fig. 3). This backward growth of the endoderm proceeds, being concentrated towards one point, at which the boundary between the ventral plate and the cell-plate of the rudimentary shell gland is marked. The changes accompanying the closure

of the blastopore and the rapid growth of the posterior region cause, as the result of slight rotation (Text-fig. 5), the establishment of the future embryonic axes, the so-called head-vesicle being gradually moved to the anterior region of the embryo, and its relative dimensions being reduced.

Even after the completion of the closure of the blastopore, the posterior region of the embryo continues to spread, and the completed gastrula, accordingly, assumes a transitory outline from a roundish to a pear shape, the thickened cell-plate of the rudimentary shell gland being indicated at the wider postero-dorsal region of the embryo (Text-fig. 4). In this stage, the posteriorly pointed end of the completely closed primitive gut fuses with the ectoderm, forming there a small anal plate. Thus, the primitive gut shows an ovoid shape, which is widened anteriorly and pointed posteriorly. Meanwhile, a blastocoelic



Text-fig. 4. Schematic representation of median, sagittal section of completed gastrula measuring  $130\ \mu$  or so in diameter.  $\times 400$ . *A* anterior end of embryo, *P* posterior end of embryo, *ap* anal plate, *hv* head-vesicle, *M* teloblastic cell-mass of mesoderm, *mes* mesenchymes, *op* oral plate, *pi* primitive gut, *sg* cell-plate of rudimentary shell gland, *vp* ventral plate, *Y* larval mesoblast.



Text-fig. 5. Diagram, illustrating relations between three stages in gastrulation.  $\times 400$ . *Ia* original polar axis of egg, *IIa* dorso-ventral axis of embryo.



space comes to appear also between the primitive gut and the ventral plate, as the effect of the subsequent growth of the latter. In this stage, the mesodermal elements show a considerable increase in number, and those transformed into the so-called mesenchymes spread also to the anterior portion of the embryo, but the main teloblastic cell-masses are yet visible at the posterior dorsal portion, each mass containing one large element. Moreover, in contact with the ventral plate, another mass of mesodermal cells is observed at the ventral side of the primitive gut. The substance of this mass may probably be detached from the teloblastic cell-masses on the occasion of the closure of the blastopore.

In conclusion, the main features of the gastrulation in *Sphaerium japonicum hiwaense* can be stated as follows: the archenteron is commenced by a small invagination of the endoderm; the closure of the blastopore is accomplished, practically, by the forward growth of the ventral plate or the postero-ventral region of the embryo; the rotation of the embryonic axes is brought out by the closure of the blastopore, by the rapid growth of the posterior region of the embryo, and, especially, by the dorsal replacement of the rudimentary shell gland (Text-fig. 5); the oral plate is formed by a stomodeal invagination of the ectoderm at the anterior portion of the blastopore, where the final closure of the blastopore had been completed; and the anal plate originates at the point, where the posterior end of the primitive gut has fused to unite with the ectoderm.

#### REMARKS ON THE GASTRULATION

Of the gastrulation of the Lamellibranchia reported by many investigators, two different types may be distinguished: the marine type and the freshwater type. The gastrulation of the former type is observable in most marine mussels and peculiarly in a freshwater mussel, *Dreissensia*. The blastocoel of the blastula of this type is originally very small or non-existent, and the endoderm consist of somewhat large cells containing yolk granules. Consequently, the formation of the archenteron is caused chiefly by an epibolic method sometimes accompanied by, but often without, invagination. Instead of this inconspicuous, endodermal invagination, another rapid and conspicuous, ectodermal invagination, which forms the shell gland at the dorsal region, begins at the same time as this process of gastrulation. A detailed account of the gastrulation of this type is given by MEISENHEIMER (1901) with regard to *Dreissensia polymorpha*.

The gastrulation of the freshwater type has been studied mainly in

the species of Sphaeriidae and Unionidae, by ZIEGLER (1885), STAUFFACHER (1894), LILLIE (1895), HERBERS (1914), and WOODS (1931). The blastula of this type has a large blastocoel, and the archenteron is formed by a true invagination of the endoderm.

In the case of the Unionidae, the thickened endoderm invaginates and forms the small sac of the archenteron. The shell gland is also formed by the invagination of the plate, consisting of large cells derived from X, and occupying the whole dorsal region of the embryo. According to LILLIE (1895) and HERBERS (1914), the invagination of the archenteron appears a little earlier than that of the shell gland, and the gastrula at this stage has two invaginated depressions, one being a small archenteron at its ventral side and the other a voluminous shell gland at the dorsal.

In the case of the Sphaeriidae, the invagination of the rudimentary shell gland is delayed to far later stage, and is never formed before the closure of the blastopore, as reported by ZIEGLER (1885), STAUFFACHER (1894), and the present writer in this paper. LILLIE (1895) states that the earlier formation and the enormous size of the shell gland in *Unio* is, of course, due to a special provision for the needs of the glochidium, which possesses an enormous shell in proportion to its bulk. At any rate, in the case of the Sphaeriidae, the primitive gut is easily distinguishable from the shell gland, owing to the delayed formation of the latter, which is a common characteristic of the embryos of the Sphaeriidae.

In the papers published before this, the closure of the blastopore was not explained positively in relation to the Sphaeriidae, and therefore, as to this point, a detailed comparison between the species of this family is impossible, except in the case of *Cyclas cornea* in which it seems to be considered by STAUFFACHER (1894) that the closure occurs at the median plane in the centre of the blastopore. But, the existence of the small endodermal invagination, and the position of the cell-plate of the rudimentary shell gland, of the head-vesicle, and of the two mesodermal masses exactly agree in every case. ZIEGLER (1885) states, in the case of *Cyclas cornea*, that each cell of the head-vesicle is large, containing the poorly chromatinized nucleus and the large vacuole of a peculiar, dim substance in the earlier stage of the gastrula, and is then converted to the flattened cell in the later stage. In the material under consideration, the head-vesicle consists of flattened elements from the beginning of the gastrulation (Pl.-fig. 1).

In conclusion, the blastoporal closure, due to the forward growth of the ventral plate, even if this may not be the case in *Cyclas cornea*, and

the embryonic topography, mentioned already in regard to the determination of the future dorso-ventral axis as due mainly to the rapid growth of the posterior region of the embryo, are recognized by many investigators as the common characteristics of the Lamellibranchia. The results as observed by the present writer in the case of *Sphaerium japonicum* are in good agreement with those observed in the case of most Lamellibranchia.

#### OBSERVATION ON THE FETAL LARVA

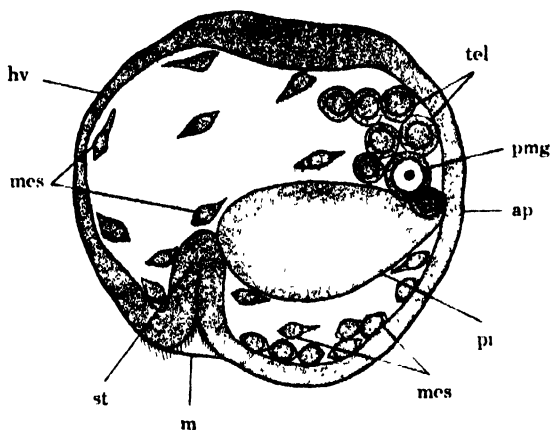
Soon after the complete closure of the blastopore, the stage of the 'fetal larva'<sup>1</sup> begins. This fetal larva of the species under consideration, which can move only a little within the marsupial sac of the mother, is quite different in its structure from the so-called 'veliger', and continues to develop until the stage of small, but adult form is reached, without any sign of metamorphosis. It is difficult, therefore, in this case, to distinguish it from the post-embryonal stage, which commences with the glochidial stage in the Unionidae, and which is the stage after metamorphosis of the veliger in most Lamellibranchia.

In the progress of the development after completion of the gastrulation, the embryo under discussion assumes gradually a pear shape which has the cell-plate of the rudimentary shell gland at the wider dorsal and the ventral plate at the narrower ventral region (Text-fig. 6). In the next stage, the extension of the ventral plate advances more to the ventral side, forming a ventral bulge, and causes an elongation of the dorso-ventral axis of the embryo. On the other hand, the cell-plate of the rudimentary shell gland at the dorsal region undergoes an invagination (Text-fig. 7), and then everts again (Text-fig. 8). After this eversion of the shell gland, the embryo assumes a mushroom-shape, this indicating the real formation of the shell over the surface of the shell gland, as the 'cuticular cap' (Text-fig. 9 and Pl.-fig. 10). The present writer here gives the definition, merely for the purposes of the present study, that the fetal larva is the stage from the completion of the gastrulation to the formation of this rudimentary shell, and has described the differentiations in the ectoderm, in the mesoderm, and in the endoderm occurring during this stage in the arrangement of this order.

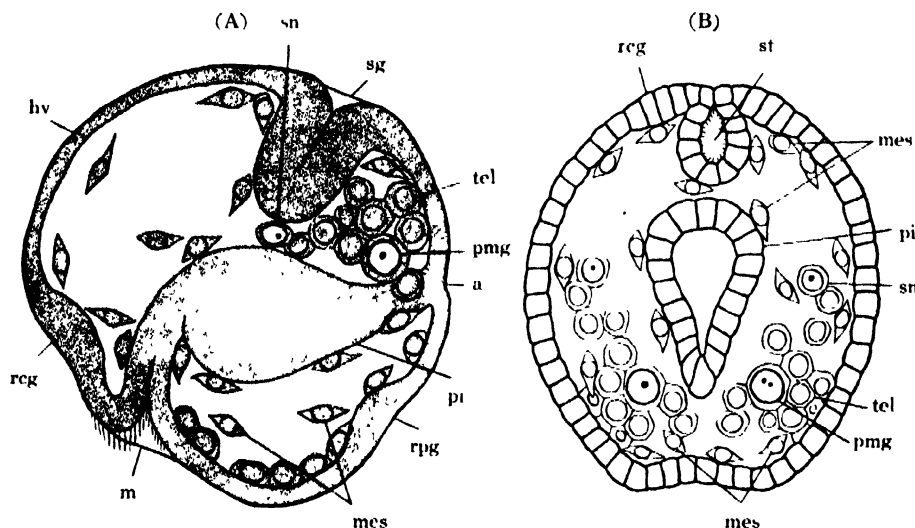
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<sup>1</sup> The 'fetal larva' is the new term used by myself to express the stage from the completion of gastrulation to the formation of the rudimentary shell, because of its being reared in the marsupial sac of the mother, as distinguished from the term 'young larva' used by LILLIE (1895) for the corresponding stage of *Unio*, from completed gastrula to glochidium.

**ECTODERM.** After the gastrulation process has been completed, the cell-plate of the rudimentary shell gland acquires the dorsal position of the embryo owing to the successive growth of its posterior region; and the head-vesicle, which consists of flattened cells, becomes much reduced relatively to the anterior portion of the embryo (Text-fig. 6). One of the remarkable changes in the ectoderm, during this stage, is the es-



Text-fig. 6. Schematic representation, illustrating optical, median, sagittal section of fetal larva, just forming stomodeum  $\times 350$ . *ap* anal plate, *hv* head-vesicle, *m* mouth, *mes* mesenchymes, *pi* primitive gut, *pmg* primordial germ cell, *sg* rudimentary shell gland, *st* stomodeum, *tel* teloblastic cell-mass of mesoderm.

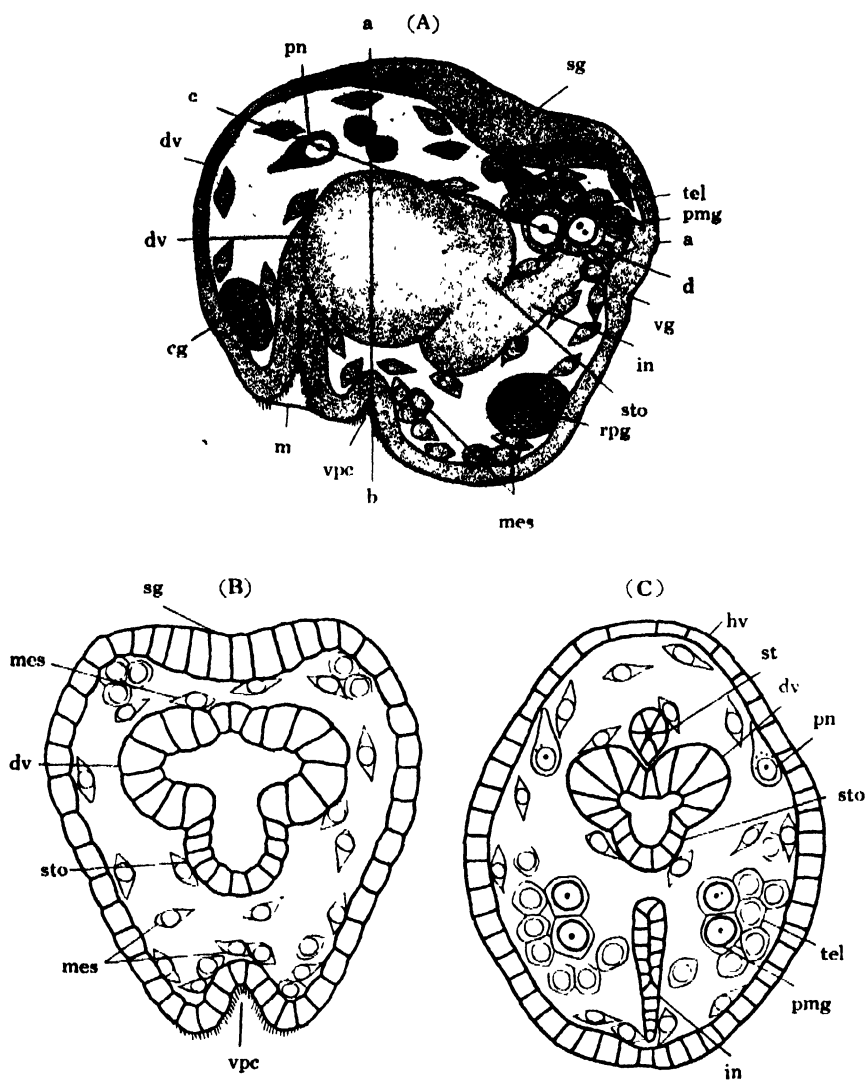


Text-fig. 7 Schematic representations, illustrating optical, median, sagittal section (A), and frontal section through mouth and teloblastic cell-masses of mesoderm (B), of fetal larva in stage of invagination of shell gland.  $\times 350$ . *a* anus, *hv* head-vesicle, *m* mouth, *mes* mesenchymes, *pi* alimentary canal, *pmg* primordial germ cell, *rcg* cerebral ganglion, *rpg* combined rudiment of pedal ganglion and byssus gland, *sg* shell gland, *sn* supposed nephroblast, *st* stomodeum, *tel* teloblastic cell-mass of mesoderm.

tablishment of the stomodeum. The stomodeal invagination deepens considerably with the progression of the ventral growth, and is changed into a tubular lumen, being transferred to the antero-ventral portion of the embryo. The external aperture of this invagination is the mouth, and the tubular lumen the stomodeum (Text-fig. 6). Fine cilia are observed on the inner surface of the stomodeal lining, especially distinct on the upper (originally anterior) rim of the mouth.

Before long, the rudimentary shell gland shows the invagination above mentioned, which is directed antero-ventrally and approaches near the anterior portion of the primitive gut (Text-fig. 7 and Pl.-fig. 5). LILLIE (1895) reports, in the case of *Unio*, that the invaginated lumen of the shell gland is filled with a transparent, refringent substance. In the case of *Sphaerium japonicum biwaense*, however, no substance was observable in the invaginated lumen. At the same time, the ciliated stomodeum opens internally to the antero-dorsal end of the primitive gut and, in the posterior region, the anal plate is perforated, and forms a small, external aperture which is the anus (Text-fig. 7 and Pl.-fig. 6).

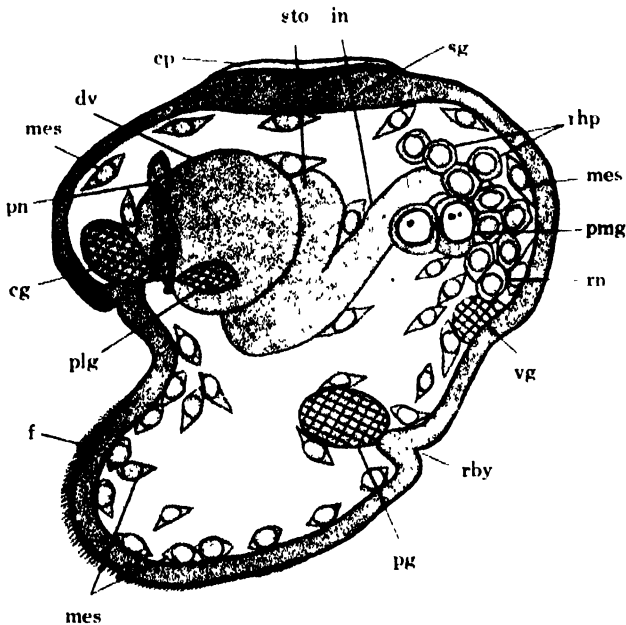
In the progress of the development, the cave-like depression of the shell gland is everted again, and, during this eversion of the shell gland, three ectodermal differentiations occur (Text-fig. 8): 1) the antero-lateral walls just opposite to the mouth indicate the paired thickenings as the rudiments of cerebral ganglia (Pl.-figs. 7 and 10); 2) in the postero-ventral wall of the ventral plate appear also paired thickenings, each of which is the combined rudiment of the pedal ganglion and the byssus gland (Pl.-fig. 7); and 3) an ectodermal depression furnished with fine cilia is formed on the ventral portion just beneath the mouth (Text-fig. 8 and Pl.-fig. 7). ZIEGLER (1885) states, in the case of *Cyclas cornea*, that the ciliated upper rim of the mouth corresponds to the upper lobe of the vestigial velum and the ventral ectodermal depression beneath the mouth corresponds to the lower lobe. This view appears to be reasonable when compared with the trochophore of *Polygordius*. This trochophore has two ciliary rings, prototroch and metatroch. According to WOLTERECK (1904), the cilia of the metatroch originate at first on the ventral and then extend to the dorsal side. Generally, in the Lamellibranchia, it is evident that the velum corresponds to the prototroch of the trochophore. Therefore, in the case of the Sphaeriidae, the cilia on the upper rim of the mouth is a vestige of the prototroch, and the post-oral cilia in the ventral depression may be that of the metatroch. Thus, it may be considered that the fetal larva of the present species under discussion expresses



Text-fig. 8. Schematic representations, illustrating optical, median section (A), transverse section through *ab* (B), and frontal section through *cd* (C).  $\times 350$ . *a* anus, *cg* cerebral ganglion, *dv* digestive diverticulum, *hv* head-vesicle, *in* intestine, *m* mouth, *mes* mesenchymes, *pmg* primordial germ cell, *pn* element of protonephridium, *rpg* combined rudiment of pedal ganglion and byssus gland, *sg* shell gland, *st* stomodaeum, *sto* stomach, *tel* teloblastic cell-mass of mesoderm, *vg* visceral ganglion, *vpc* ciliated ventral depression.

a vestigial veliger.

After eversion, the shell gland is observed as a conspicuous roof of cell-plate, occupying the larger portion of the dorsal surface and being covered by a delicate cuticular cap (Text-fig. 9 and Pl.-figs. 8 and 9). According to LILLIE (1895), such a cap-shaped cuticle is derived from the above-mentioned, refringent secretion of the invaginated shell gland. Another remarkable change in the ectoderm, in this later stage, is the formation of the foot. A rapid bulging of the ventral plate towards the ventral side occurs as if it were forming the stalk of a mushroom (Text-fig. 9). This ventral plate becomes ciliated, at present, and shows more or less a property of expansion and contraction; and is henceforth called the foot of the embryo. Meanwhile, further development of the cerebral and pedal ganglia, the formation of the byssus gland, the appearance of pleural and visceral ganglia, and the lateral compression of the body are



Text-fig. 9 Schematic representation, illustrating optical, median, sagittal section of fetal larva in its final stage.  $\times 300$ . *cg* cerebral ganglion, *cp* cuticular cap of shell, *dv* digestive diverticulum, *f* foot, *in* intestine, *mes* mesenchymes, *pg* pedal ganglion, *plg* pleural ganglion, *pmg* primordial germ cell, *pn* protonephridium, *rby* rudiment of byssus gland, *rhp* original cells of heart and pericardium, *rn* original cells of kidney, *sg* shell gland, *sto* stomach, *vg* visceral ganglion.

to be observed. The cells of the rudimentary cerebral ganglia are condensed into two compact cell-masses, which begin to connect with each other just in front of the stomodeum (Pl.-fig. 11). The paired ectodermal thickenings of the rudimentary pedal ganglia at the postero-ventral region of the foot begin to invaginate, and form the rudiments of the byssus gland (Pl.-fig. 12), at the tops of which the rudimentary pedal ganglia differentiate into paired compact masses, the rudiments of byssus gland remaining at their original position in the foot (Pl.-fig. 13). The rudiments of the visceral ganglia are produced at the ventral body wall beneath the anus, though they are very obscure in this stage (Text-fig. 9 and Pl.-figs. 14 and 19), and those of the pleural ganglia at the lateral body wall behind the cerebral ganglia (Text-fig. 9 and Pl.-fig. 15). On the other hand, the bilateral compression of the embryo takes place in the portion just beneath the level of the mouth and anus, resulting in a more distinct constriction of the foot, while the subsequent expansion of the dorsal portion continues and causes the formation of the future mantle fold.

**MESODERM.** After the gastrulation, the spindle-shaped mesenchyme cells increase in number. ZIEGLER (1885) states that, in the case of *Cyclas cornea*, these mesenchymes are derived from the ectoderm. The writer is of opinion that the mesenchyme cells are mainly derived from the larval mesoblastic cell-mass, resulting from its disintegration, because, in this stage, they are scattered and no compact mesodermal cell-mass is seen in the anterior region of the embryo (Text-figs. 6 and 7). LILLIE (1895) states regarding *Unio* that the divisions of the larval mesoblastic cells bear no definite relation to the embryonic axes, but are irregular and mesenchymal in their lack of coördination.

The paired cell-masses of the teloblastic mesoderm are observed in contact with the body wall from the posterior end of the body to some anterior extent, showing a slight elongation, even though the typical mesodermal bands, as seen in the Annelida, are not formed. Each cell-mass contains, invariably, a large, round element, which is the direct descendant of M, having a large, vesicular nucleus with one or two distinct nucleoli, and is distinguished accurately, in its cytological structures, from the other co-existent descendants of M, which are at present an indifferent cell-mass, consisting of small elements, each of which has a compact nucleus (Pl.-fig. 16). In the stage of eversion of the shell gland, this large cell, which has already been the primordial germ cell, divides equally on either side of the body (Text-fig. 8 and Pl.-fig. 17). After



this division, these large cells continue to undergo distinct specialization as the germ cells. Woods (1931 and '32) states, in the case of *Sphaerium striatinum*, that these two primordial germ cells on either side of the body now enter a relatively long period of inactivity, which continues until they have reached their position in the definite gonad.

In the later stage of the fetal larva, on either side of the body, some elements of the teloblastic cell-mass are detached towards the rudimentary intestine and show signs of arrangement surrounding the latter. These members are the original cells of the heart and pericardium. The other members detached on the ventral side of the primordial germ cells begin to show a tubular arrangement, indicating the rudiments of the kidney (Pl.-fig. 18). MEISENHEIMER (1901) states, regarding *Cyclas*, that the main portion of kidney originate from the posterior ectodermal ingrowth, while Woods (1931), in the case of *Sphaerium striatinum*, states that not only the kidney, but also the gonad, heart, and pericardium originate from paired, indifferent, teloblastic cell masses, which are derived from the 4d or M in the cleavage stage.

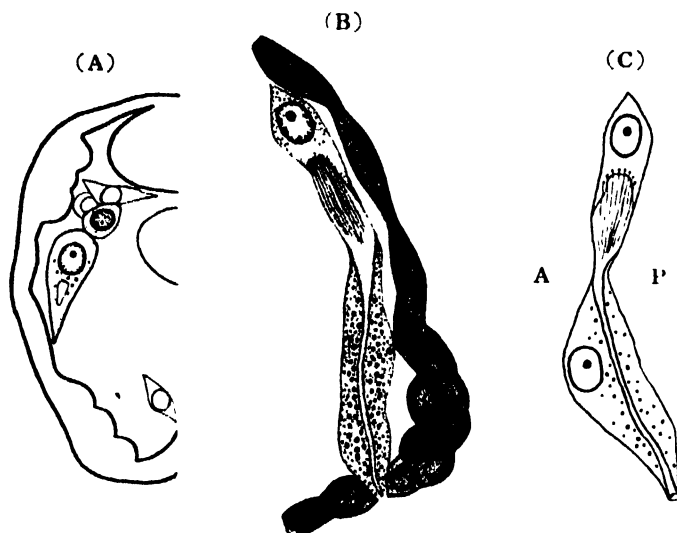
On the other hand, a transformation to the myocytes from some of the mesenchymal elements is seen, especially in the posterior, anterior, and foot region of the fetal larva (Pl.-fig. 19).

**PROTONEPHRIDIUM.** The exact origin of the protonephridium has not been determined, even though it appears to originate from the mesoblastic cells, as in the case of *Physa fontinalis* reported by WIERZEJSKI (1905). In this paper merely the preliminary observation is described.

In the same stage as that shown in Text-fig. 8, a peculiar cell is seen inside the anterior body wall on either side of the body. It is furnished with a vesicular nucleus which contains a distinct nucleolus, and with loosely granulated cytoplasm, which shows a purplish colour stronger than do the others when stained by Mallory's connective tissue staining method. This peculiar cell seems to be descendant of an element at the most anterior end of each teloblastic cell-mass, seen in the stages of Text-figs. 6 and 7, and to be correspondent with the nephroblast reported by WIERZEJSKI (1905).

In the later stage of the fetal larva, on either side of the body, two or three cells are found inside the body wall behind the head-vesicle. They are the descendants of this supposed nephroblast. The most anterior cell among them begins to extend its plasm towards the body wall, indicating its differentiation to the excretory tubule (Text-fig. 10 a), and contains distinct granules and a vacuole. This vacuole opens soon to the

exterior (Pl.-figs. 22-25). In the stage shown in Text-fig. 9, the fully developed protonephridium is observable on either side of the larva.



Text-fig. 10. Schematic representations, illustrating development of protonephridium: (A) protonephridium in transverse section of body parallel to stomodeum in stage of Text-fig. 8.  $\times 700$ . (B) protonephridium in transverse section in stage of Text-fig. 9.  $\times 1000$ . (C) outside view of fully developed right protonephridium.  $\times 1000$ . A anterior, P posterior.

This protonephridium is composed of a typical flame cell and an excretory tubule (Text-fig. 10 b and c and Pl.-fig. 26).

**ENDODERM.** When the shell gland begins to invaginate, the stomodeum communicates with the antero-dorsal end of the primitive gut. Meanwhile, the posteriorly pointed end of the primitive gut opens to the exterior, and develops to the anus. Thus, the larval alimentary canal consists, at present, of three parts, *viz.* the anterior tubular stomodeum, the middle spacious stomach, and the posterior narrow intestine. In the species under consideration, the appearance of the crystalline style is in the far later stage of development. Occasionally, in the spacious lumen of the rudimentary stomach of this stage, one or two large round cells with compound nuclei are found (Pl.-fig. 27). These round cells are the elements, without doubt, fallen from the walls of the marsupial sac, and may be explained as the larval nutriment (OKADA, 1935 b). They are found even in an incomplete archenteron, though rarely and exceptionally, and also shown near the larval mouth (Pl.-fig. 28). At the same time, the outer

surface of the alimentary canal becomes lined here and there with some of the mesenchymes.

In the stage of eversion of the shell gland, paired evaginations are found on the lateral walls of the rudimentary stomach. The cells constituting the evaginated walls are large, and have a granulated cytoplasm taking a little more purplish colour than do the others when stained by Mallory's connective tissue staining method (Pl.-fig. 30). These evaginations are the rudiments of the digestive diverticula. And further, in the later differentiation of the alimentary canal, the constriction between the rudimentary stomach and intestine becomes more marked owing to the dorsal bending of the latter (Text-fig. 9). At the same time, cilia begin to be observed on the walls of the stomach proper (Pl.-fig. 30).

#### REMARKS ON THE FETAL LARVA

**EXTERNAL FORM.** It is well known that the completed gastrula of most Lamellibranchia develops to the so-called trochophore and veliger. The trochophore of *Yoldia limatula* has an apical tuft and three rings of cilia (DREW, 1899). Most of the other members, viz. *Anomia*, *Cardium*, *Dreissensia*, *Entovalva*, *Modiola*, *Montacuta*, *Mytilus*, *Ostrea*, *Parapholas*, *Solen*, *Teredo*, *Xylotrya*, etc., pass through the young stage with their velum and bivalved shell.

On the other hand, the non-existence of the veliger is found in Unionidae, Sphaeriidae, and in a few others. *Nucula delphinodonta* passes through a stage of degenerated trochophore with feeble cilia on all its surface (DREW, 1899). In the Unionidae, extremely fine cilia are observed on the surface of the embryo. LILLIE (1895) states that these cilia are not supposed to represent a vestigial velum but are homologous with those on ventral surface of other Molluscan embryos. In the Sphaeriidae, as already stated in reference to the species under discussion, the extremely vestigial prototroch and metatroch are seen during the stage of the fetal larva. The absence of velum in these three forms above-mentioned may depend on the viviparous habit of rearing their young. In reality, as already stated in comparison with other Lamellibranchia, *Sphaerium japonicum* indicate signs of secondary direct development in which the metamorphosis is indistinct, merely the earlier development of the foot and the vestigial signs of the head region having been shown.

**INTERNAL ORGANIZATION.** The free swimming life of the veliger and the parasitic life of the glochidium are not carried out in the case of the

fetal larva under discussion, and, therefore, neither the larval organs of the veliger, such as the apical plate and larval adductor muscle, nor those of the glochidium, such as the thread gland and larval mantle, are found in this fetal larva.

The protonephridium, which is the common, larval organ of the Lamellibranchia with the exception of the Unionidae, however, is the only remarkable larval organ found in the fetal larva of the present species, notwithstanding its vestigial structures. STAUFFACHER (1898) observed this organ in the larva of *Cyclas cornea*, but the result of his observation is not in agreement with that of the present writer. The result of my observation looks like that of *Dreissensia polymorpha* reported by MEISENHEIMER (1901).

During the stage of the fetal larva, the rudiments of the adult organs are well in agreement with those of other Lamellibranchia in their development, especially in comparison with MEISENHEIMER's description of *Dreissensia polymorpha*. It may, however, be noted here that the earlier development of the alimentary canal, the delayed formation of the embryonic shell, and the distinct occurrence of comparatively large, primordial germ cells seem to be special characters of the present material.

#### SUMMARY

1) The results of observations and remarks on the gastrula and fetal larva of *Sphaerium japonicum biwanense* MORI are reported in detail in this paper.

2) The archenteron is formed by a small endodermal invagination.

3) The closure of the blastopore is caused by a forward growth of the ventral plate.

4) The oral plate is formed between the stomodeal invagination and the anterior end of the primitive gut.

5) The anal plate is formed between the posteriorly extended end of the primitive gut and the ectoderm at the junction of the ventral plate with the cell-plate of the rudimentary shell gland.

6) The original embryonic axes are transferred to the larval axes by a slight rotation caused by the forward growth of the ventral plate and the dorsal replacement of the rudimentary shell gland.

7) The stage from the completion of gastrulation to the formation of rudimentary shell is now for the first time termed the stage of the 'fetal larva.'

8) The cilia at the upper rim of the mouth are homologous to the prototroch and those at the ventral depression beneath the mouth may be homologous to the metatroch.

9) The rudiments of the cerebral and pedal ganglia develop earlier than do those of pleural and visceral ganglia.

10) The rudiments of the gonad, kidney, heart and pericardium originate from the teloblastic cell-mass of the mesoderm.

11) The protonephridium is found on both sides of the larva

12) The earlier differentiation of the alimentary canal, the delayed formation of the embryonic shell, and the distinct occurrence of comparatively large, primordial germ cells seem to be special characters of *Sphaerium japonicum biwaense*.

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## ABBREVIATIONS IN PLATES

<i>A</i> anterior end	<i>my</i> myocyte
<i>P</i> posterior end	<i>och</i> original cells of heart and pericardium
<i>a</i> anus	<i>on</i> original cells of kidney
<i>bp</i> blastopore	<i>pg</i> pedal ganglion
<i>by</i> byssus gland	<i>pi</i> archenteron or primitive gut
<i>cc</i> cuticular cap	<i>plg</i> pleural ganglion
<i>cgc</i> commissure between cerebral ganglia	<i>pmg</i> primordial germ cell
<i>cg</i> cerebral ganglion	<i>pn</i> original cell of protonephridium
<i>cs</i> cilia of stomach	<i>rpg</i> rudiment of pedal ganglion and byssus gland
<i>dv</i> digestive diverticulum	<i>sg</i> shell gland
<i>f</i> foot	<i>st</i> stomodeum
<i>fc</i> flame cell of protonephridium	<i>sto</i> stomach
<i>ext</i> excretory tubule of protonephridium	<i>tel</i> teloblastic cell-mass of mesoderm
<i>hv</i> head-vesicle	<i>vg</i> visceral ganglion
<i>idm</i> indifferent cell-mass of teloblastic mesoderm	<i>vp</i> ventral plate
<i>int</i> intestine	<i>vpc</i> ciliated ventral depression
<i>lrc</i> large round cell, fallen from wall of marsupial sac	<i>wm</i> wall of marsupial sac
<i>m</i> mesenchymes	<i>Y</i> larval mesoblast
<i>ml</i> mouth of larva	

## EXPLANATION OF PLATE I.

- 1) Median section of gastrula forming archenteron.  $\times 400$ .
- 2) Median section of gastrula in stage of closing blastopore.  $\times 400$ .
- 3) Median section of gastrula having closed blastopore.  $\times 400$ .
- 4) Ventral portion of median section of completed gastrula.  $\times 400$ .
- 5) Median section of fetal larva having invaginated shell gland.  $\times 350$ .
- 6) Median section of fetal larva in stage of 5), to show primitive gut.  $\times 350$ .
- 7) Median section of fetal larva in stage of everting shell gland.  $\times 300$ .
- 8) Median section of fetal larva in its final stage  $\times 250$ .
- 9) Section of shell gland in final stage of fetal larva, to show presence of cuticular cap.  $\times 900$ .
- 10) Section perpendicular to stomodeum in stage of fetal larva shown in Text-fig. 8, to show rudiments of cerebral ganglia.  $\times 700$ .
- 11) Section perpendicular to stomodeum in final stage of fetal larva, to show cerebral ganglia.  $\times 700$ .
- 12) Section of foot in final stage of fetal larva, to show rudiments of byssus gland.  $\times 900$ .

## EXPLANATION OF PLATE II.

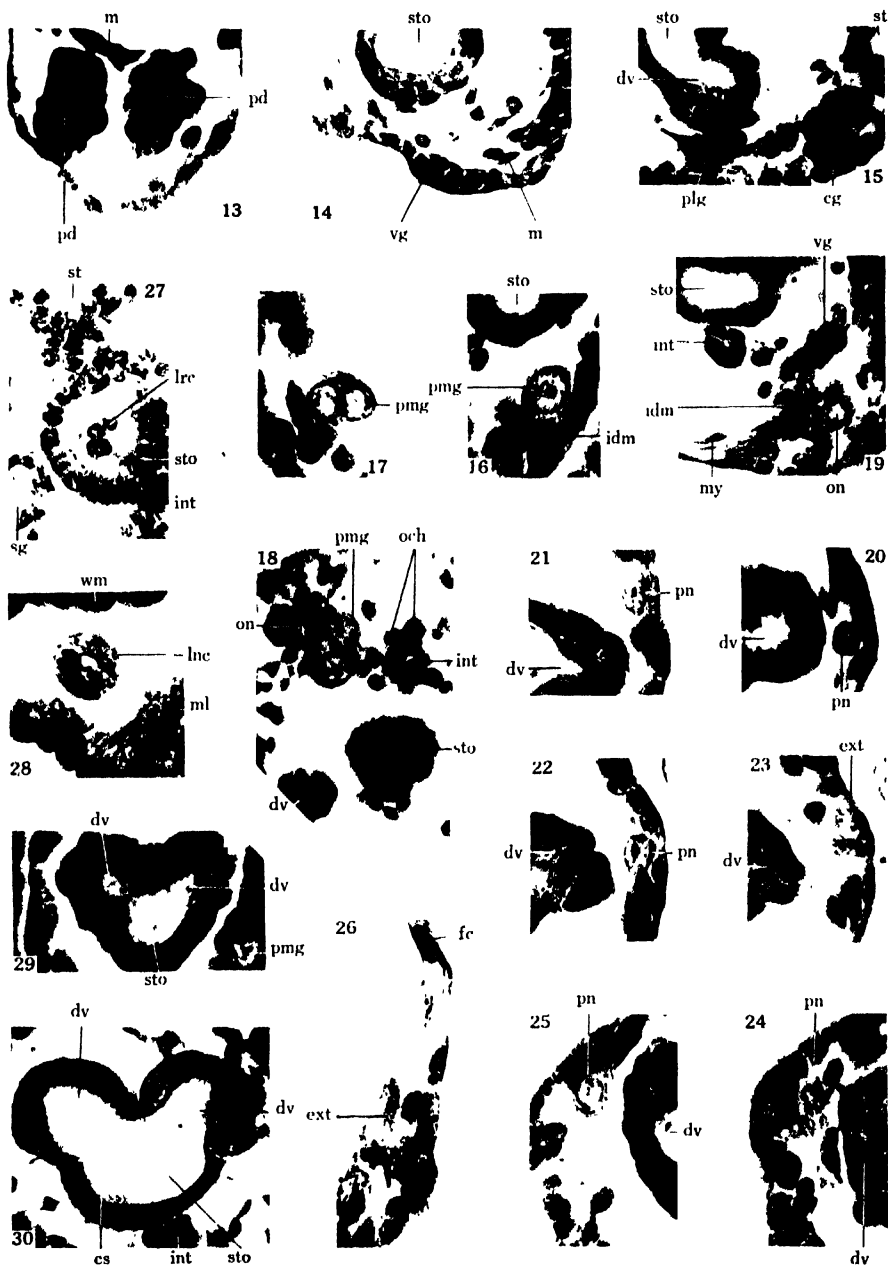
- 13) Section of foot in final stage of fetal larva, to show pedal ganglia.  $\times 700$ .
- 14) Median section of posterior region of body in final stage of fetal larva, to show rudimentary visceral ganglion.  $\times 350$ .
- 15) Horizontal section of antero-lateral region of body in final stage of fetal larva, to show rudimentary pleural ganglion.  $\times 500$ .
- 16) Horizontal section of posterior region of body in final stage of fetal larva shown in Text-fig. 8, to show primordial germ cell before equal division.  $\times 700$ .
- 17) Horizontal section of posterior region of body in stage of fetal larva shown in Text-fig. 8, to show primordial germ cells after equal division.  $\times 700$ .
- 18) Horizontal section of posterior region of body in final stage of fetal larva, to show original cells of heart, pericardium, and kidney.  $\times 500$ .
- 19) Horizontal section of posterior region of body in final stage of fetal larva, to show myocytes.  $\times 500$ .
- 20)–23) Horizontal sections of antero-lateral region of body in stage of fetal larva shown in Text-fig. 8, to show development of protonephridium.  $\times 700$ .
- 24)–25) Horizontal sections of antero-lateral region of body in later stage of fetal larva, to show development of protonephridium.  $\times 700$ .
- 26) Cross section perpendicular to antero-posterior axis of body in final stage of fetal larva, to show fully developed protonephridium.  $\times 700$ .
- 27) Median section of rudimentary stomach in stage of fetal larva shown in Text-fig. 7, to show large round cell with compound nuclei  $\times 700$ .
- 28) Large, round cell with compound nuclei near mouth of fetal larva.  $\times 900$ .
- 29) Cross section perpendicular to antero-posterior axis of body in stage of fetal larva shown in Text-fig. 8, to show relation between rudimentary stomach and digestive diverticula.  $\times 700$ .
- 30) Cross section perpendicular to antero-posterior axis of body in final stage of fetal larva, to show relation between stomach and digestive diverticula.  $\times 700$ .



K. OKADA: *Sphaerium japonicum*: Gastrula and Fetal Larva.







K. OKADA: *Sphaerium japonicum*: Gastrula and Fetal Larva.



# DIMENSIONAL, MORPHOLOGICAL AND ZOOGRAPHICAL STUDY OF JAPANESE CRABS OF THE GENUS *TELMESSUS*<sup>1)</sup>

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(With seven figures)

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The carapace of *Telmessus* WHITE 1846<sup>2)</sup> is nearly pentagonal, the length being shorter than the width. The front is divided into three lobes, viz. a median lobe, which is subdivided into four teeth, and two lateral lobes forming the pre-orbital spines. Either lateral margin of the carapace is armed with six teeth, including the post-orbital spine. The surface of the spinous legs and carapace is densely covered with short bristles.

The species of this genus are edible, and are found commonly in a shallow sea from the littoral line to a depth of nearly 20 fathoms of the North Pacific Ocean, their habitat, southwards, being limited to the coast of California of North America and of the northern part of Japan.

*Telmessus cheiragonus* (TILESIIUS 1812)<sup>3)</sup> is a species widely distributed in the North Pacific Ocean. Besides this, in 1858, STIMPSON distinguished a new species, *Cheiragonus acutidens*, from an examination of specimens from Hakodate. In 1879, MIERS adopted this view. In 1892, BENEDICT gave a detailed description of the two species, *Telmessus cheiragonus* and *Telmessus acutidens*, and proposed a new genus, *Erimacrus*, to include *Platycorystes isenbeckii* = *Cheiragonus isenbeckii* of BRANDT. He thus reached the conclusion that the genus *Telmessus* comprises only two species, *T. cheiragonus* and *T. acutidens*, the latter of which is different from the former in having longer and slenderer teeth on the lateral margin of the carapace, a small intermediate tooth at the posterior base of the epi-

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<sup>1)</sup> The publication of this paper is due to the kindness of Prof. Dr. E. NOMURA, to whom the writer wishes to express his sincere thanks.

<sup>2)</sup> *Telmessus* WHITE 1846 = *Platycorystes* BRANDT 1848, *Cheiragonus* BRANDT 1851.

<sup>3)</sup> *Telmessus cheiragonus* (TILESIIUS 1812) = *Cancer cheiragonus* TILESIIUS 1812 (1815), *Telmessus serratus* WHITE 1846, *Platycorystes cheiragonus* BRANDT 1851, *Cheiragonus hippocarcinoides* BRANDT 1851, *Cheiragonus cheiragonus* ORTMANN 1894 (part).

branchial spine, and, especially, in the epibranchial spine itself. Moreover, it is noted that the latter species has four denticles on the anterior margin of the epibranchial spine, while the former has five.

In 1894, however, ORTMANN considered *Telmessus acutidens* to be a synonym of *Telmessus cheiragonus* in his investigation of specimens (2 males and 3 females) obtained from Tôkyô Bay; from de Castry Bay, Primorskaya; and from Japan (locality uncertain). This opinion was adopted by PARISI (1916), BALSS (1922), and by YOKOYA (1928).

On the other hand, American investigators, namely HOLMES (1900), SCHMITT (1921) and RATHBUN (1930), continue to distinguish *T. acutidens* from *T. cheiragonus*.

Whether the two species of *Telmessus* are distinct, as American investigators maintain, or are really one and the same, as European investigators insist, was a question which was assigned to me. As a fact, the specimens of *Telmessus* from Mutsu Bay are of the *acutidens*-type, while those from Saghalien are of the *cheiragonus*-type not only morphologically but also zoogeographically. Moreover, the *cheiragonus*-type is more widely distributed, viz. from the west coast of North America to Muroran and the neighbourhood on the east coast of Asia, while the distribution of the *acutidens*-type is more restricted, viz. from about Muroran to Tôkyô Bay<sup>1)</sup>. My intention, therefore, was to study and compare specimens from both places, Saghalien and Mutsu Bay, and to solve the question, if possible.

The specimens from Saghalien were collected at Maoka on the west coast by the kindness of Mr. G. YAMADA. Maoka is the warmest locality in Saghalien, and, if the intermediate types<sup>2)</sup> between *cheiragonus* and *acutidens* existed, it was presumed that they would be found in such a neighbourhood. The specimens from Mutsu Bay were sent by the good offices of Dr. HÔZAWA and Dr. KOKUBO. Moreover, I was able to examine specimens from Kaiba Island, from Hokkaido and from Chôsen through the kindness of Messrs. KIMURA, NISHIMURA, TAKAGI, IMAI, MOTOKI, KAWADA, KITaura, KAMITA, and MAKI. I have, here, to express my sincere thanks to the gentlemen above-mentioned.

<sup>1)</sup>The distribution of the *cheiragonus*-type follows RATHBUN (1902, 1930), and that of the *acutidens*-type follows BENEDICT (1892), and STIMPSON et RATHBUN (1907). The descriptions by ORTMANN (1894), PARISI (1916) and by BALSS (1922) probably concern both types.

<sup>2)</sup>"Most of the crabs that belong to this genus," states PARISI (1916, p. 189), "have the intermediate characters between the two species."

## MEASUREMENT

The linear dimensions of 110 males and 97 females, from Saghalien, and of 47 males and 57 females, from Mutsu Bay, were measured with callipers and compasses. The determination of the growth curve was

TABLE 1.  
*Male from Saghalien.*

Carapace length (L) in mm.	Average width of carapace (W) in mm.	Average width of carapace excl. epibranchial (W') in mm.	W-W'	Average length of epibranchial (A) in mm.	Average width of epibranchial (B) in mm.	Carapace length (L) in mm.	Average width of carapace (W) in mm.	Average width of carapace excl. epibranchial (W') in mm.	W-W'	Average length of epibranchial (A) in mm.	Average width of epibranchial (B) in mm.
29.0	35.7	25.7	10.0	5.2	5.5	58.0	69.0	54.0	15.0	9.0	11.0
29.3	37.4	27.0	10.4	5.3	6.0	59.0	71.0	55.0	15.0	9.5	12.0
31.5	39.6	28.4	11.2	5.6	6.0	59.5	71.7	55.7	15.0	9.4	11.3
32.6	41.7	30.0	11.7	6.0	6.2	59.6	73.0	57.0	16.0	10.0	12.0
33.0	41.4	30.4	11.0	5.5	6.5	60.0	73.3	57.0	16.3	10.0	12.5
36.4	45.7	34.0	11.7	6.0	8.0	60.5	74.8	58.7	16.1	10.2	12.6
37.8	48.0	36.0	12.0	6.3	7.3	60.7	76.0	58.0	18.0	9.5	12.0
39.4	50.0	36.0	14.0	7.0	7.8	61.3	73.0	57.0	16.0	9.5	12.0
40.0	49.5	37.0	12.5	6.5	7.4	61.5	74.0	57.5	16.5	9.5	11.7
40.7	52.0	38.0	14.0	7.0	8.0	62.0	75.0	57.2	17.8	9.2	13.0
41.3	52.5	38.0	14.5	7.6	9.0	62.2	75.0	59.0	16.0	9.0	12.0
41.7	52.0	38.0	14.0	7.0	8.0	62.3	75.0	57.5	17.5	9.5	12.5
42.5	54.1	39.6	14.5	7.6	8.7	63.0	76.7	59.0	17.7	9.7	12.8
46.7	58.3	43.4	14.9	7.5	9.8	63.5	74.3	58.5	15.8	9.5	12.0
47.0	57.4	44.0	13.4	7.3	9.2	64.0	76.7	59.2	17.5	10.6	12.3
47.6	58.6	44.2	14.4	7.5	9.2	64.5	76.0	60.0	16.0	9.2	12.5
47.8	60.2	45.0	15.2	9.0	10.0	65.0	78.3	60.2	18.1	11.0	13.0
48.5	61.0	45.0	16.0	9.0	10.0	65.5	77.2	60.8	16.4	10.0	12.0
49.5	61.0	46.0	15.0	8.7	9.7	65.7	79.0	62.0	17.0	11.0	13.0
50.0	63.0	47.5	15.5	8.2	10.4	66.0	81.5	61.5	20.0	11.2	13.0
50.2	61.5	46.0	15.5	8.0	10.0	66.5	79.6	61.5	18.1	9.5	12.0
50.3	61.0	46.5	14.5	8.5	10.0	66.7	79.0	61.8	17.2	11.0	13.0
50.5	61.5	47.0	14.5	8.6	9.7	67.0	79.6	61.7	17.9	10.5	12.5
51.0	61.5	47.0	14.5	8.0	9.3	67.2	80.0	63.5	16.5	10.5	12.5
51.4	62.2	47.2	15.0	8.0	10.3	67.5	80.8	63.1	17.7	10.0	12.5
52.2	64.0	48.0	16.0	8.7	10.0	67.8	80.0	62.8	17.2	11.0	13.0
52.5	64.5	49.0	15.5	9.0	10.0	68.0	81.5	63.2	18.3	10.6	12.3
54.0	65.5	50.5	15.0	9.4	10.9	68.3	82.0	64.5	17.5	10.8	12.0
54.8	66.5	51.0	15.5	9.0	11.5	68.5	80.3	64.0	16.3	11.2	13.0
55.0	68.0	51.0	17.0	9.0	11.0	69.0	85.0	65.5	19.5	11.0	13.3
55.3	69.0	52.0	17.0	9.0	11.0	69.3	81.5	65.0	16.5	11.5	14.0
55.5	68.4	53.0	15.4	9.0	12.0	70.0	85.7	65.6	20.1	11.7	13.7
56.0	69.5	51.0	18.5	10.3	11.2	70.5	84.5	64.5	20.0	11.5	13.0
56.5	69.0	52.0	17.0	9.0	11.0	71.0	83.8	66.0	17.8	10.7	13.2
56.7	70.0	54.0	16.0	9.5	11.7	71.2	85.3	67.0	18.3	11.0	14.0
57.0	70.4	53.2	17.2	9.2	10.3	72.0	86.0	67.0	19.0	11.0	13.0
57.4	71.0	54.0	17.0	9.0	12.0	73.0	85.0	66.0	19.0	11.0	14.0
57.5	71.0	54.0	17.0	11.0	12.0	75.7	88.0	68.0	20.0	11.0	14.0

carried out with the assistance of Mr. I. HAMAI, to whom I wish to express my deep sense of gratitude.

In the records of measurements,  $L$  denotes the shortest distance between the anterior end of the frontal lobe and the posterior margin of the carapace.  $W$  the distance from the outer end of the left epibranchial spine to that of the right, and  $W'$  the distance from the anterior base of the left epibranchial spine to that of the right. Therefore  $W-W'$  is the sum of the lengths of the left and right epibranchial spines. Consequently,  $W/L \times 100$  is the percentage ratio of the width of the carapace, including

TABLE 2.  
*Female from Saghalien.*

Carapace length ( $L$ ) in mm.	Average width of carapace ( $W$ ) in mm.	Average width of carapace excl. epibranchial ( $W'$ ) in mm.	$W-W'$	Average length of epibranchial ( $A$ ) in mm.	Average width of epibranchial ( $B$ ) in mm.	Carapace length ( $L$ ) in mm.	Average width of carapace ( $W$ ) in mm.	Average width of carapace excl. epibranchial ( $W'$ ) in mm.	$W-W'$	Average length of epibranchial ( $A$ ) in mm.	Average width of epibranchial ( $B$ ) in mm.
26.2	32.4	25.0	7.4	4.0	4.8	44.0	54.0	41.5	12.5	7.5	8.7
32.0	40.3	30.0	10.3	5.2	6.2	44.2	53.6	42.6	11.0	7.0	8.0
32.7	40.0	30.0	10.0	5.0	6.0	44.5	54.5	42.0	12.5	8.0	9.0
33.0	41.0	30.2	10.8	5.5	6.0	45.0	54.5	43.0	11.5	7.0	8.5
33.5	40.0	30.0	10.0	5.0	6.8	46.0	55.1	43.3	11.8	6.6	8.3
34.0	41.8	31.1	10.7	5.7	6.6	46.4	57.0	44.0	13.0	7.0	9.0
34.3	41.7	31.5	10.2	6.0	7.0	47.0	57.5	44.0	13.5	7.4	9.1
35.0	43.5	32.0	11.5	5.7	7.0	48.0	57.6	44.0	13.6	7.1	9.2
35.2	43.6	33.3	10.3	6.0	7.0	48.5	59.0	46.0	13.0	7.3	9.0
36.0	44.3	33.5	10.8	6.0	7.0	49.0	60.5	48.5	12.0	7.5	10.0
37.0	45.3	35.2	10.1	5.8	7.0	49.2	57.2	47.0	10.2	6.2	9.0
37.4	46.2	35.5	10.7	5.4	7.2	49.3	59.3	47.0	12.3	7.0	9.0
37.6	47.0	36.0	11.0	6.0	7.0	50.0	59.3	45.0	14.3	7.2	9.5
37.7	47.0	36.0	11.0	6.5	8.0	50.2	61.0	48.0	13.0	7.2	9.5
38.0	46.4	35.7	10.7	6.2	7.0	50.6	60.0	47.5	12.5	7.6	9.7
38.4	47.3	36.0	11.3	6.5	7.5	51.0	60.0	48.0	12.0	7.0	9.5
38.5	47.6	36.5	11.1	6.5	8.0	51.4	63.0	50.0	13.0	7.0	9.5
38.6	47.0	35.0	12.0	6.0	7.5	52.0	62.0	49.5	12.0	8.0	10.0
39.0	48.0	37.0	11.0	6.2	7.5	53.0	61.5	51.0	13.5	8.0	10.5
39.5	48.4	37.2	11.2	6.0	7.0	53.5	65.0	50.0	15.0	8.0	11.0
40.0	48.5	37.3	11.2	6.7	7.7	54.0	66.0	51.4	14.6	7.5	10.0
40.2	48.0	36.5	11.5	6.5	7.2	55.0	64.2	51.2	13.0	7.7	10.2
40.5	51.6	40.0	11.6	6.5	7.5	55.4	66.0	52.0	14.0	8.0	11.0
40.8	49.5	37.0	12.5	7.0	8.0	56.0	67.0	53.2	13.8	8.0	10.0
41.0	50.5	38.2	12.3	7.2	8.2	57.0	68.0	55.0	13.0	9.0	11.0
41.2	49.0	38.0	11.0	7.0	8.0	57.5	66.2	53.5	12.7	7.0	10.0
41.5	50.0	38.0	12.0	7.0	8.0	57.6	64.0	50.3	13.7	8.0	10.0
41.7	50.4	39.5	10.9	6.3	7.4	57.8	68.0	55.0	13.0	8.0	10.0
42.0	50.5	38.3	12.2	6.9	8.1	58.0	70.5	55.0	14.5	8.2	11.0
42.8	52.8	41.5	11.3	6.5	8.0	59.2	74.0	57.2	16.8	9.0	12.0
43.0	52.3	40.2	12.1	6.7	7.9	62.4	77.0	61.0	16.0	9.0	12.0

the two epibranchial spines, to the length of the carapace, and  $W \cdot W'/W' \times 100$  the percentage ratio of the sum of the lengths of the epibranchials to the width of the carapace, excluding the epibranchials. Furthermore,  $A$  denotes the length at the anterior margin, and  $B$  the width at the base of the epibranchial spine. Therefore,  $B/A \times 100$  is the percentage ratio of the width at the base to the length of the epibranchial spine.

The results of measurement were tabulated and are shown in Tables 1-4. In reference to these tables, it ought to be explained here that the value of  $W \cdot W'/2$ , or the assumed length of an epibranchial spine, differs from that of  $A$ , or the length of the epibranchial spine, on its anterior margin. This inequality is due mainly to the shape of this spine. If the anterior margin of the epibranchial spine were perpendicular to the longitudinal axis of the body, both values would be equal (Fig. 1 a), but such cases are rare. In most cases,  $A$  is larger than  $W \cdot W'/2$ , because  $W$  or  $W'$  is a perpendicular line and  $A$  is a lateral line starting from the vertex of a triangle (Fig. 1 b).

TABLE 3.  
*Male from Mutsu Bay.*

Carapace length ( $L$ ) in mm.	Average width of carapace ( $W$ ) in mm.	Average width of carapace excl. epibranchial ( $W'$ ) in mm.	$W \cdot W'$	Average length of epibranchial ( $A$ ) in mm.	Average width of epibranchial ( $B$ ) in mm.	Carapace length ( $L$ ) in mm.	Average width of carapace ( $W$ ) in mm.	Average width of carapace excl. epibranchial ( $W'$ ) in mm.	$W \cdot W'$	Average length of epibranchial ( $A$ ) in mm.	Average width of epibranchial ( $B$ ) in mm.
29.5	38.2	24.8	13.8	7.0	5.3	53.5	71.0	47.0	24.0	12.0	11.0
30.0	41.0	25.6	15.4	7.7	6.0	54.0	71.0	47.0	24.0	13.0	10.5
30.5	42.6	25.7	16.9	9.0	6.5	55.0	69.3	47.3	22.0	11.2	10.5
32.0	42.2	28.0	14.2	7.2	6.2	56.0	72.0	49.6	22.4	11.3	11.0
34.0	45.3	28.6	16.7	8.8	6.8	56.5	75.5	50.8	23.7	14.0	12.0
35.0	47.3	30.0	17.3	9.0	7.0	59.2	77.6	52.0	25.6	14.5	12.0
35.8	46.5	30.0	16.5	8.3	6.2	59.3	80.0	52.0	28.0	15.0	12.0
37.0	47.7	32.2	15.5	8.3	7.7	59.4	77.5	52.0	25.5	14.0	12.0
37.4	51.0	33.0	18.0	9.0	7.0	60.0	78.4	55.0	23.4	12.4	11.5
37.7	51.8	33.0	18.8	10.2	7.7	62.7	78.0	55.0	23.0	12.0	12.0
38.0	50.2	33.0	17.2	8.7	8.0	64.0	83.0	56.0	27.0	14.0	12.0
40.7	53.0	37.0	16.0	9.0	8.0	65.5	84.3	59.0	25.3	14.5	13.0
42.2	55.2	36.0	19.2	10.0	7.5	66.5	87.0	59.0	28.0	14.8	13.0
46.0	61.6	42.0	19.6	11.0	9.3	67.0	86.2	56.5	29.7	15.0	13.0
46.7	64.0	41.2	22.8	12.0	8.2	72.0	91.5	62.5	29.0	14.5	14.0
47.5	61.7	42.3	19.4	11.0	9.0	72.5	90.0	67.0	23.0	13.0	14.0
48.0	65.0	42.0	23.0	12.0	10.0	76.2	102.6	78.0	24.6	15.0	15.0
51.2	68.0	44.0	24.0	12.5	10.0	83.0	104.5	74.0	30.5	16.0	16.0
52.2	68.0	46.0	22.0	12.0	11.0	85.0	108.0	77.0	31.0	17.0	17.0
53.0	72.0	46.0	26.0	13.0	11.0						



TABLE 4.  
*Female from Mutsu Bay.*

Carapace length (L) in mm.	Average width of carapace (W) in mm	Average width of carapace excl. epibranchial (W') in mm	W-W'	Average length of epibranchial (A) in mm.	Average width of epibranchial (B) in mm.	Carapace length (L) in mm.	Average width of carapace (W) in mm.	Average width of carapace excl. epibranchial (W') in mm.	W-W'	Average length of epibranchial (A) in mm.	Average width of epibranchial (B) in mm.
27.4	39.5	23.0	16.5	8.2	6.2	59.3	79.0	52.5	26.5	13.3	13.0
27.7	38.5	23.3	15.2	7.6	5.5	60.0	78.5	54.0	24.5	13.0	11.8
30.0	41.0	26.0	15.0	7.7	5.7	63.0	81.0	56.0	25.0	14.0	13.0
30.4	41.2	26.0	15.2	7.8	6.0	63.2	81.0	56.0	25.0	13.0	12.5
34.5	46.1	30.0	16.4	8.2	7.2	64.3	81.5	57.0	24.5	12.5	12.2
35.2	46.0	30.0	16.0	8.5	7.0	66.5	87.0	60.0	27.0	14.0	13.0
37.0	49.0	32.5	16.5	9.0	6.8	68.0	84.0	62.0	22.0	12.0	14.0
41.0	53.0	35.0	18.0	10.0	8.3	69.0	85.0	64.0	21.0	11.5	13.5
44.0	57.5	38.0	19.5	10.0	8.8	70.0	87.5	63.0	24.5	13.0	14.0
44.5	58.7	39.2	19.5	10.5	8.7	71.0	85.6	65.0	20.6	12.0	14.0
44.7	60.0	40.0	20.0	10.0	8.0	72.0	87.5	66.0	21.5	12.0	14.0
45.0	57.0	40.0	17.0	9.0	8.0	72.3	87.5	66.0	21.5	11.0	13.0
46.0	60.2	41.0	19.2	10.0	9.0	75.0	89.0	69.5	19.5	12.0	13.0
47.0	63.5	42.0	21.5	11.5	9.5	76.0	91.0	70.0	24.0	13.0	14.0
47.3	63.6	42.0	21.6	11.5	10.0	77.5	94.0	69.0	25.0	14.0	14.2
47.7	63.0	42.0	21.0	10.8	9.0	78.0	94.0	72.0	22.0	13.5	14.5
50.0	65.0	46.0	19.0	10.5	10.0	79.0	95.6	72.5	23.1	13.0	15.0
51.0	67.0	45.0	22.0	12.0	10.5	81.0	95.5	71.0	24.5	12.2	13.0
52.5	68.5	46.3	22.3	12.5	10.0	82.2	99.7	78.0	21.7	12.0	13.5
54.0	68.0	49.0	19.0	10.0	11.0	83.0	99.0	74.0	25.0	14.5	15.0
55.0	72.0	49.0	23.0	12.7	10.5	84.5	103.5	76.0	27.5	14.0	16.0
57.0	73.0	49.0	24.0	12.0	11.0	86.5	100.0	78.0	22.0	11.0	15.0
57.2	74.0	52.0	22.0	12.0	11.5	88.0	106.0	78.0	28.0	15.0	16.0
58.8	76.0	51.0	25.0	13.0	12.0						

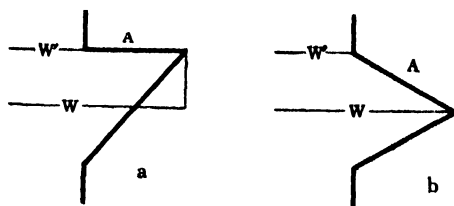


Fig. 1. Schematic representation of epibranchial spine, to illustrate relation between  $W-W'/2$  and  $A$ . *a* case of  $W-W'/2 = A$ , *b* case of  $W-W'/2 < A$ .

THE GROWTH RELATION BETWEEN THE WIDTH ( $W$ ) AND THE LENGTH ( $L$ ) OF THE CARAPACE The results of calculation are as follows<sup>1)</sup>: —

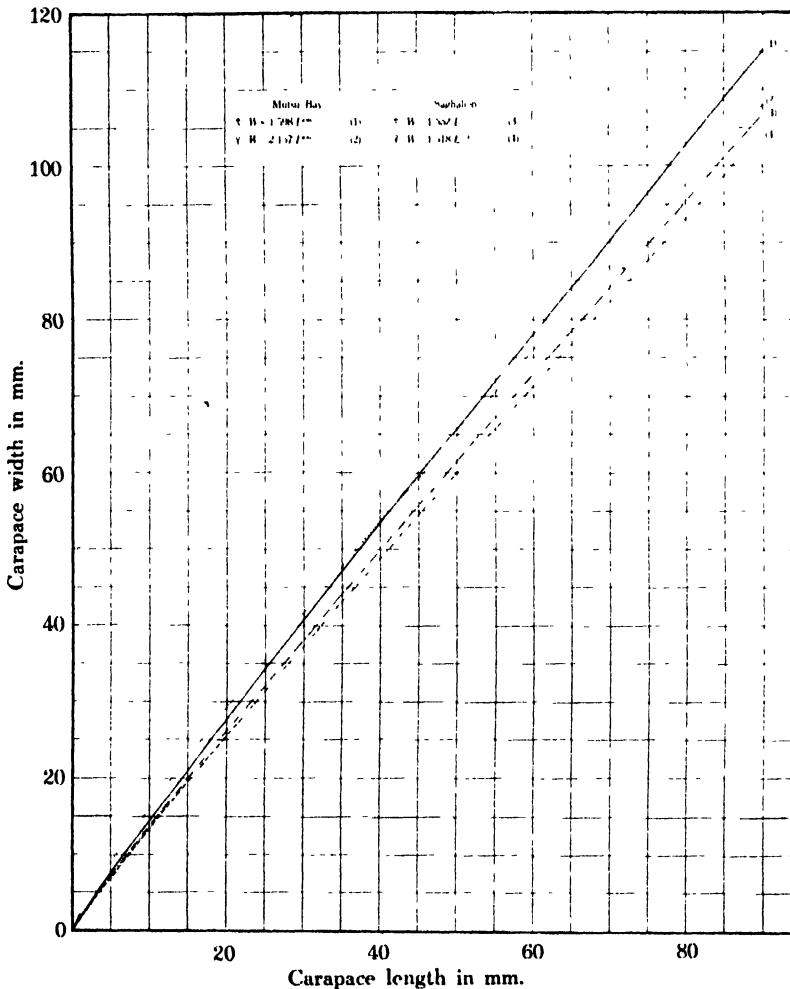


Fig. 2. Growth curves of *Telmessus* crabs from Mutsu Bay (1 and 2) and from Saghalien (3 and 4).

<sup>1)</sup>The following relations had already been given by SASAKI (1928) in relation to the specimens from Mutsu Bay:

$$\begin{array}{ll} \text{Male} & C = 1.393 L^{0.95} \quad \text{or} \quad C = 1.346 L^{0.96} \\ \text{Female} & C = 1.372 L^{0.95} \quad \text{or} \quad C = 1.324 L^{0.96} \end{array}$$

where  $C$  denotes the carapace width and  $L$  the carapace length. The differences between SASAKI's figures and mine are due to his exclusion of the dimensions of the median frontal lobe and of the epibranchial spines. SASAKI's equations reveal that the linear dimensions of the carapace, excluding those of the spines, show a similar growth relation in both male and female.

	Saghalien	Mutsu Bay
Male	$W = 1.552 L^{0.94}$	$W = 1.598 L^{0.97}$
Female	$W = 1.518 L^{0.94}$	$W = 2.157 L^{0.85}$

As shown in Fig. 2, the growth relation between the width and the length of the carapace of the male, in the specimens from Saghalien, shows a similar tendency to that in the female, but in those from Mutsu Bay, this relation is quite different from that in the female.

The crab of the genus *Telmessus* has a carapace, which is broader than its length. Therefore,  $W/L \times 100$  is invariably larger than 100 (Table 5).

Naturally, even in the crabs having the same length of carapace, several variations are found in the percentage ratio. But, generally, this ratio becomes smaller with the increase of the length of the carapace, i. e. the smaller the size of the crab the greater the width of the carapace, and the larger the size of the crab the narrower the width, in comparison with the length, of the carapace.

As seen in Table 5, the specimens from Mutsu Bay show a wider variation and a higher value of percentage ratio than those from Saghalien. In reality, most of the males and nearly half the females from Mutsu Bay show a larger value of percentage ratio than the largest among those from Saghalien.

In the case of those having the same value for  $W/L \times 100$ , the specimens from Mutsu Bay show a carapace length much larger than that of those from Saghalien. In reality, the specimens from Mutsu Bay, the percentage ratio of which is less than 128, invariably show a carapace length

TABLE 5.

*Frequency of the percentage ratio of the width to the length of the carapace.*

$W/L \times 100$	Frequency			
	Saghalien		Mutsu Bay	
	Male	Female	Male	Female
112		1		
113		1		
114		1		
115		3		
116	2	2		1
117	6	2		
118	7	3		2
119	13	10		3
120	14	14		4
121	12	15		3
122	11	13		2
123	11	12		1
124	8	11	4	2
125	9	6	2	2
126	9	3	1	3
127	6	1	1	1
128	2		1	1
129			3	3
130			10	8
131			4	6
132			6	4
133			2	
134			3	3
135			2	1
136			3	2
137			3	
138				
139				1
140			1	
141				
142			1	
143				
144				
145				1

larger than 60 mm., just twice the length of or more of the specimens from Saghalien of the same ratio.

Within the group of crabs having the same length of carapace,  $W/L \times 100$  is always larger in the specimens from Mutsu Bay than in

TABLE 6.

*Relation between the carapace length and the percentage ratio of the length of the epibranchial spines to the carapace width excluding the epibranchial spines.*

Length of carapace in mm.	$W/L \times 100$			
	Saghalien		Mutsu Bay	
	Male	Female	Male	Female
26-30	125	124	137	139
30-40	127	123	133	133
40-50	125	121	133	131
50-60	122	119	131	130
60-70	121		129	127
70-80	118		127	120
80-90			127	120
90-93			124	

those from Saghalien, and the greatest difference between the values of the percentage ratio is found in the smaller crabs 26-30 mm. in carapace length (Table 6). In other words, the specimens from Mutsu Bay always have a carapace width greater than that of the specimens from Saghalien, especially when they are young.

In the specimens from Saghalien,  $W/L \times 100$  is always greater in the male than in the female, *i. e.* the carapace width of the male is always greater than that of the female. In the specimens from Mutsu Bay, however, the carapace width of the male is narrower when small, but

TABLE 7.

*Frequency of the percentage ratio of the length of the epibranchial spines to the width of the carapace excluding the epibranchials.*

$W-W' \times 100$ $W'$	Frequency			
	Saghalien		Mutsu Bay	
	Male	Female	Male	Female
23		1		
24		3		
25	4	9		
26	6	11		
27	13	12		
28	12	9		2
29	9	10		1
30	11	11		
31	12	9		2
32	11	8	1	4
33	10	6		3
34	6	3	2	2
35	2	3		4
36	4	2		1
37	3	1		
38	2			
39	4		1	2
40	1		1	
41			1	2
42			3	3
43			5	1
44				
45			2	3
46			1	
47			2	3
48			4	
49			2	4
50				4
51			4	5
52			1	2
53			3	1
54			3	
55			4	1
56			2	
57				1
58			2	1
59				
60			2	1
66			1	
71				1

it becomes wider than that of the female, with the growth of the carapace (Fig. 2 and Table 6).

THE RELATION BETWEEN THE LENGTH OF THE EPIBRANCHIAL SPINE ( $W-W'$ ) AND THE WIDTH OF THE CARAPACE EXCLUDING THE EPIBRANCHIALS ( $W'$ ) As shown in Table 7, the specimens from Saghalien show a narrower variation and generally a smaller value of  $W-W'/W' \times 100$  than those from Mutsu Bay. In reality, the length of the epibranchial spines of both male and female specimens from Saghalien is nearly from 23% to 40% of the width of the carapace, excluding the epibranchials. In the specimens from Mutsu Bay, however, the ratio ranges from 32% to 66% in the male, and from 28% to 71% in the female.

TABLE 8.

*Relation between the carapace length and the percentage ratio of the length of the epibranchial spines to the carapace width excluding the epibranchials.*

Length of carapace in mm.	$\frac{W-W'}{W'} \times 100$			
	Saghalien		Mutsu Bay	
	Male	Female	Male	Female
26-30	39	30	58	60
30-40	36	31	55	54
40-50	34	29	50	49
50-60	31	27	49	46
60-70	29		47	40
70-80	28		40	33
80-90			40	33
90-93			34	

Naturally, even in the crabs of the same carapace length, the epibranchial spine shows variations, but in general the shorter the carapace length the greater the value of  $W-W'/W' \times 100$ , but this value becomes smaller with the lengthening of the carapace, i. e. the smaller the size of a crab the longer the epibranchials and the larger the size the shorter they become, in comparison with the width of the carapace. This phenomenon is more strongly marked in the specimens, especially in the female, from Mutsu Bay than those from Saghalien (Table 8).

Within the group of specimens having the same carapace length, the epibranchial spine is always longer in the male than in the female. But in the smaller specimens from Mutsu Bay the reverse is the case. Moreover, in the specimens from Mutsu Bay, the epibranchial spine is longer than that of the specimens of the same carapace length from Saghalien, especially in the crabs smaller than 40 mm. in carapace length. Furthermore, in the case where the percentage ratio has the same value, the length of the carapace is much larger in the specimens from Mutsu Bay than in those from Saghalien (Table 8).

The specimens from Mutsu Bay, the percentage ratio of which is less

than 40 or which show a similar percentage ratio to those from Saghalien, are invariably old and large, the carapace length measuring more than 67 mm.

THE RELATION BETWEEN THE WIDTH ( $B$ ) AND THE LENGTH ( $A$ ) OF THE EPIBRANCHIAL SPINE (Table 9). The male and female specimens from Saghalien show nearly the same frequency distribution of the values of  $B/A \times 100$ , and in both sexes  $B$  is always larger than  $A$ . Of the specimens from Mutsu Bay, however, the males show  $B$  smaller than  $A$  in 45 cases out of 17, and the females cases of  $B$  smaller than  $A$  in 34 (63%) out of 54, and cases of  $B$  larger than  $A$  only in 20 (37%). It is worthy of note here that in the specimens from Mutsu Bay, which show an epibranchial spine wider than the length, the males measure more than 72.5 mm. and the females more than 68 mm. in carapace length.

THE DIMENSIONAL DIFFERENCES OF THE TYPES As mentioned above, the *acutidens*-type from Mutsu Bay

is clearly distinguishable from the *cheiragonus* type from Saghalien in the point of dimensions, even though the form and the length of the epibranchial spine of the large specimens of the *acutidens*-type resemble those of the small specimens of the *cheiragonus* type. Therefore, at least, within the limit of the group of specimens of the same size, the present writer has, definitely, come to the conclusion that the *acutidens*-type is to be clearly distinguished from the *cheiragonus*-type

TABLE 9.

*Frequency of the percentage ratio of the width to the length of the epibranchial spine*

$B/A \times 100$	Frequency			
	Saghalien		Mutsu Bay	
	Male	Female	Male	Female
70-75			4	3
75-80			10	5
80-85			8	7
85-90			9	7
90-95			5	5
95-100			9	7
100-105	1		1	8
105-110	8	5		1
110-115	17	16		8
115-120	37	24	1	
120-125	14	16		2
125-130	25	14		1
130-135	6	10		
135-140	2	8		
140-145		4		
145-146		1		

#### EXTERNAL FEATURE

In the preceding section, the distinction between the types, *cheiragonus* and *acutidens*, has been determined, dimensionally. In the present section, the external characters, which have hitherto been described in distinguishing the species, are to be re-examined.

**THE CARAPACE** The carapace of the crabs from Mutsu Bay is rougher and more convex than that of those from Saghalien. According to BENEDICT (1892) and STIMPSON (1858, 1907), the dorsal surface of the carapace of *T. acutidens* is overlaid with tubercles, while that of *T. cheiragonus* is covered with granules, and in its posterior region they show linear arrangements. In reality, if we remove the bristles of the carapace, the difference between the species is clearly observable. The so-called tubercles of the carapace of *acutidens*-type are larger and more pointed than those of the *cheiragonus*-type.

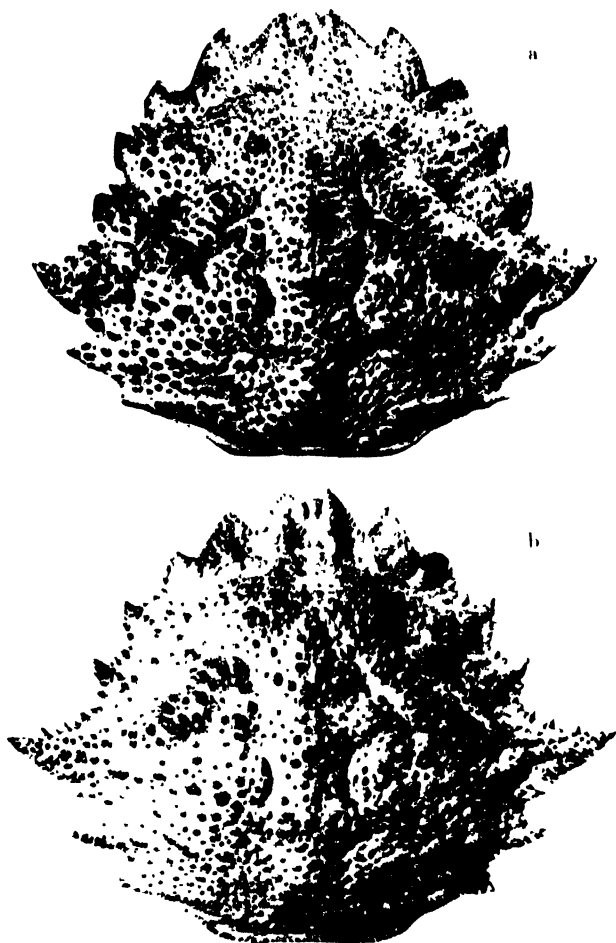


Fig 3. Carapace of *Telmessus cheiragonus* from Saghalien (a) and of *T. acutidens* from Mutsu Bay (b). Natural size.

The denticles on the anterior margin of the epibranchial spine of the *cheiragonus*-type are 4-5 in number, while those of the *acutidens*-type are more than 5.

The denticles on the posterior margin of the lateral teeth of the *cheiragonus*-type are small, and, when viewed from the ventral side of the body, all the teeth show smooth posterior lines, while those of the *acutidens*-type are projected outwards and are directly observable from the ventral side, and, especially, a remarkable denticle is situated just at the posterior base of the epibranchial spine.

In the *cheiragonus*-type, the four teeth at the anterior end of the front are triangular and are of nearly equal size, but in the *acutidens*-type the inner two teeth are considerably smaller than the two outer ones.

**THE FEMALE ABDOMEN** As one characteristic of the genus *Telmessus*, it is stated by BENEDICT (1892) that the lateral margin of the sixth abdominal segment of the female is broadly incised to such an extent that the genital orifice is exposed on either side of the body. Although the genital orifice is invariably exposed in both types, in the crabs of the *acutidens*-type the incision is only slight, while in those of *cheiragonus*-type it is prominent, sometimes forming a semicircle (Fig. 4).

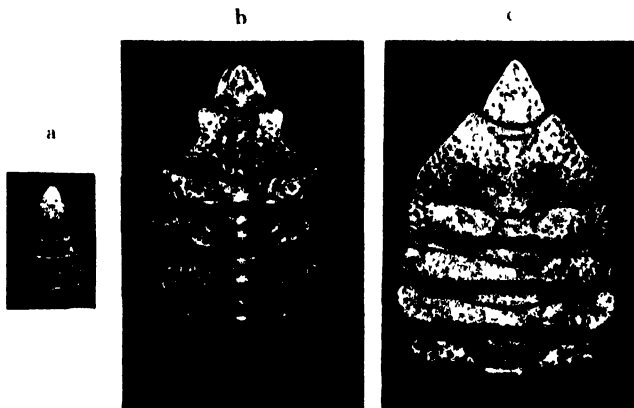


Fig. 4. Female abdomen of *Telmessus cheiragonus* from Saghalien (a and b) and of *T. acutidens* from Mutsu Bay (c). Natural size

The lateral margins and the central transverse ridge of the second and of the third abdominal segment of the crab of the *acutidens*-type are overlaid with small tubercles, linearly arranged, while those of each abdominal segment of the *cheiragonus*-type are smooth being without any tubercles.



Either pleuritic process of the third abdominal segment of the *cheiragonus*-type is protruded antero-laterally, but that of the *acutidens*-type is protruded simply anteriorly.

**THE MALE ABDOMEN** The abdominal segments from the third to the fifth are fused together, and the anterior two-thirds of the fused segment forms an elongated triangle together with the sixth and seventh abdominal segments. Either pleuritic process of the third abdominal segment is located, just at the base of this triangle. The lateral margins of the pleuritic processes of the *acutidens*-type run parallel with each other, while those of the *cheiragonus*-type diverge antero-laterally (Fig 5).

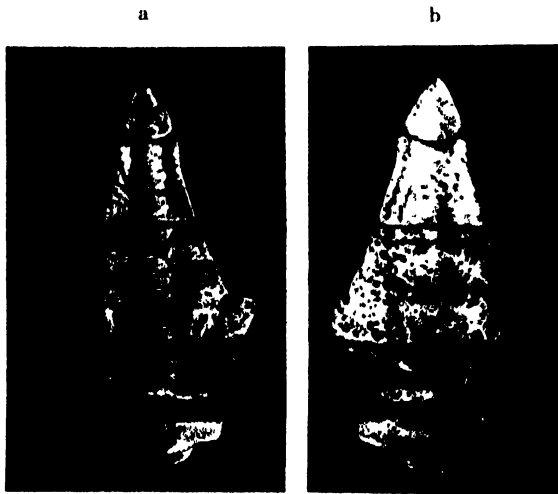


Fig. 5. Male abdomen of *Telmessus cheiragonus* from Saghalien (a) and of *T. acutidens* from Mutsu Bay (b). Natural size.

**THE FEMALE GENITAL ORIFICE** This is large and round, and opens near either lateral margin of the sixth sternal plastron, where the third pereopod is articulated. In females of more than 37 mm. in carapace length, collected in Mutsu Bay in May and in Saghalien in June and July, the genital pore is closed by a big plug of dirty rag-like substance. When this is removed, the round and deep genital pore opens underneath. The plugged portion of the genital pore is white, lustrous and funnel-shaped.

The periphery of the genital orifice of the female specimens of the *acutidens*-type is depressed ovoidally. Near the narrower end of the ovoidal depression the genital pore opens, and the plastron at its peri-

phery shows no peculiarities. The plastron at the periphery of the genital pore of the female of the *cheiragonus*-type, and especially its inner portion is thickened and surrounds the genital pore, forming a semicircular bank-

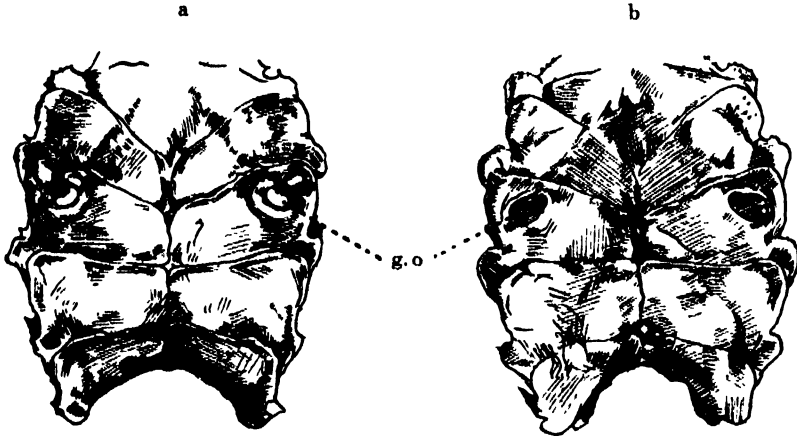


Fig. 6. Female sternum of *Telmessus cheiragonus* from Saghalien (a) and of *T. acutidens* from Mutsu Bay (b). g.o.—genital orifice.

like elevation, and just opposite to this elevation, there is a tubercle. This tubercle together with the semicircular elevation has an ear-like appearance.

#### DISTINCTION OF THE SPECIES

The *Telmessus* crabs from Saghalien exactly correspond in structure with *Telmessus cheiragonus* as described by BENEDICT (1892) and RATHBUN (1930), and those of Mutsu Bay with *Telmessus acutidens* as described by STIMPSON (1858, 1907) and BENEDICT (1892). In conclusion, the present writer, in support of the view held by American investigators, has definitely come to the conclusion that *T. cheiragonus* and *T. acutidens* are distinct species, this opinion being based on the recognition of several dimensional and morphological differences between these two types of *Telmessus* crabs. The present writer here maintains that the intermediate types between the two species, the existence of which was suggested by PARISI (1916), have never been found in any single case, in the course of his study, with the exception of cases of the existence of the same dimensions of epibranchial spines in small specimens of *T. cheiragonus* as in larger specimens of *T. acutidens*. Therefore, the genus *Telmessus* comprises, at present, the following two species:

1. *Telmessus cheiragonus* (Tilesius)

Nom. Jap., Kurigani.

RATHBUN, 1930, p. 150, text-fig. 21, and its synonymy.

Distribution: from Bering Sea southwards to the northern part of California and to Siberia, Saghalien and Hokkaido.

Distribution in Japan: Horomushiro, Kurile Islands (Science Museum, August, 1931); Shana, Yotorup, Kurile Islands (RATHBUN); Nemuro (RATHBUN, KAWATA); Kushiro (TOMARUME); Muroran (RATHBUN, IMAI); Abashiri (TAKAGI); Saghalien (BRASHNIKOW, URITA).

2. *Telmessus acutidens* (Stimpson)

Nom. Jap. nov., Igakurigani.

STIMPSON, 1858, p. 40; MIERS, 1879, p. 36; BENEDICT, 1892, p. 228, pl. 26, fig. 1; RATHBUN, 1902, p. 28; STIMPSON et RATHBUN, 1907, p. 88, pl. 12, fig. 3.

*Cheiragonus cheiragonus*, ORTMANN, 1894, p. 420 (part).

*Telmessus cheiragonus*, PARISI, 1916, p. 189 (part); BALSS, 1922, p. 98 (part); YOKOYA, 1928, p. 771.

Distribution: Muroran (RATHBUN, IMAI); Hakodate (STIMPSON, Science Museum); Kaiba Island, Saghalien (KIMURA); Wakanai (KITAURA); Rumoe (MOTOKI); Oshoro (RATHBUN); Otaru (TERAZAKI<sup>1)</sup>); Yoichi (NISHIMURA); Mutsu Bay (TERAZAKI, YOKOYA); Nambu (PARISI); Ayukawa (RATHBUN); Sendai Bay (TERAZAKI); Fukura, Chiba Prefecture (TERAZAKI); Kanagawa and Yokohama (TERAZAKI, BENEDICT); Yokosuka (BENEDICT); Fuzan, Chinkai Bay, Tôyei, and Yokuchi Island, Chôsen (KAMITA); off Kyobun Island, Chôsen (34° 33' 50" N., 127° 46' E., depth 44 m., temperature 8° 51' C., February 14, Chôsen Fishery Institute).

Finally the writer maintains that the generic diagnosis of *Telmessus*, viz. that the lateral margin of the sixth abdominal segment of the female are broadly incised, need not be taken into account.

## DISTRIBUTION

Along the Pacific coast of North America, the northern limit of the

<sup>1)</sup> The identification of TERAZAKI's specimen with *Telmessus acutidens* is due to his description that "on the lateral margin of the carapace, there is a very small spine directly posterior to the longest spine, and that arranged next to this is a medially large spine, and, next, a medially small spine."

distribution of *Telmessus cheiragonus* is Port Clarence, Alaska (nearly 65° N.), and its southern limit is the northern part of California (nearly 40° N.), nearly in coincidence with the North Sub-division of the Pacific Ocean according to the Zoogeographic Divisions of F. DAHL (1925). Along the coast of Asia, according to RATHBUN, the northern limit is the Commander Islands and the southern limit the northern part of Japan. I am, however, of opinion that the southern limit of this species is Muroran and the neighbourhood<sup>1)</sup> along the Pacific Ocean, and the Sôya Strait and the neighbourhood<sup>2)</sup> along the Sea of Japan. Kaiba Island<sup>3)</sup>, which belongs, administratively, to Saghalien, is actually separated from the main island of Saghalien by a sea depth of more than 200 m. This Island ought to belong zoogeographically rather to the system of Rishiri and Reibunshiri<sup>4)</sup> which are washed by the Tsushima Current. The southern limit of *T. cheiragonus* along the Siberian coast appears to the present writer to be from Vladivostok and the neighbourhood to the boundary between Chôsen and Siberia. But he has no positive proof for this view.

The northern limit of the distribution of *Telmessus acutidens* is exactly the southern limit of that of *T. cheiragonus*<sup>5)</sup>. Along the Pacific coast of Japan, this species is distributed from Muroran to Tôkyô Bay. Along the Japanese coast facing the Sea of Japan, it is distributed from Wakanai

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<sup>1)</sup>RATHBUN reports both *T. cheiragonus* and *T. acutidens* from Muroran. IMAI collected at Muroran 21 males and 11 females of *T. cheiragonus* and 3 males of *T. acutidens* out of 35 specimens. All the 9 specimens of TOMARUME's collection from Kushiro, a little far north-east of Muroran, were *T. cheiragonus*, while the collection from Hakodate were all *T. acutidens*.

<sup>2)</sup>All the specimens of KITaura's collection from Wakanai on the south coast of Sôya Strait were *T. acutidens*, and those from Saghalien *T. cheiragonus*.

<sup>3)</sup>6 specimens of KIMURA's collection from Kaiba Island contained 4 of *T. cheiragonus* and 2 of *T. acutidens*.

<sup>4)</sup>*Brachynotus sanguineus*, etc. inhabiting a warm sea are found at Kaiba Island.

<sup>5)</sup>MIFERS (1879) states that 44° 27' N., which passes near Nemuro, is the northern limit of *T. acutidens*. His statement in distinguishing *T. acutidens* from *T. serratus*, which is a synonym of *T. cheiragonus*, runs as follows: "This species is separated from the *Telmessus serratus* of the western American coast by a very slight characteristic, the somewhat longer and slenderer teeth of the lateral margins, particularly the third tooth." His specimen, however, was immature being only 3/4 inch long, and in such specimens it is quite natural to find longer and slenderer lateral spines in comparison with those of larger specimens of *T. cheiragonus*, as mentioned already. Therefore, I doubt whether MIFERS' specimen was actually *T. acutidens* or not. From my direct observation, KAWATA's collection at Nemuro (1 female and 4 males), TAKAGI's at Abashiri (1 female and 2 males) and TOMARUME's at Kushiro (7 females and 2 males) were all *T. cheiragonus*, and specimens of both *T. cheiragonus* and *T. acutidens* begin to be found at Muroran.

and Kaiba Island to Mutsu Bay<sup>1)</sup>. In Chôsen, it is made clear by KAMITA's collections that Chinkai Bay and neighbourhood is the southern limit of the distribution of *T. acutidens*. This limit exactly coincides with the boundary between the eastern and southern areas in the marine divisions of the ichthyological distribution of Chôsen discussed by Mr. KEITARO UCHIDA (1935).

According to RATHBUN (1930), *T. cheiragonus* is only found along the littoral line to a depth of 17 fathoms, but it is also obtained even at a depth of 20 fathoms in the neighbourhood of Ôdomari. According to BENEDICT (1892), *T. acutidens* is collected to a depth of 8 fathoms, but it is also obtained even to a depth of 44 m. Therefore, it is most probable that both species can live in the sea from the littoral line to a depth of about 20 fathoms.

If we compare the distribution of *T. cheiragonus* and of *T. acutidens* with the isothermal lines on the surface of the sea water in September, in which the temperature is at its maximum, it may be found that the surface isothermal line of 20°C. and that of 25°C., respectively, nearly coincide with the limitation of the distribution of *T. cheiragonus* and of *T. acutidens*. In fact, on the Pacific Ocean side, the surface isothermal line of 20°C. in September of a normal year begins in Volcano Bay, Hokkaido, and runs eastwards to the open sea, and on the Japan Sea side, it begins in Sôya Strait and runs towards the boundary between Chôsen and Siberia passing near Kaiba Island. These lines exactly coincide with the southern limit of the distribution of *T. cheiragonus*. The surface isothermal line of 25°C. begins on the Pacific Ocean side near Kujûkuri-Hama, in Chiba prefecture, and runs towards the open sea, and on the Japan Sea side it begins near the boundary between the Prefectures of Akita and Aomori and runs towards Quelpart Island along the southern coast of Chôsen. These lines nearly coincide with the southern limit of the distribution of *T. acutidens*. Nearly the same conditions, as mentioned above, occur even in the surface isothermal line of 1°C. and of 10°C. in February.

From the above facts, it appears to the present writer that there may

<sup>1)</sup> Mr MASAO NAKAMURA wrote to the present writer that specimens of *T. acutidens* were collected off Niigata Prefecture. But the writer lost the opportunity of directly observing them, because of their destruction by fire. Mr. KANZAEMON KIKUCHI wrote to the present writer that *T. acutidens* is not found in Toyama Bay. If these facts are true, it may be thought that the southern limit of the distribution of *T. acutidens* on the west coast of Japan is off Niigata Prefecture.

be some limitation in the biotic distribution in the shallow sea near the Sôya Strait connecting with the boundary of Chôsen. In fact, on taking other species of crabs into consideration, viz. *Brachynotus penicillatus*, *Brachynotus sanguineus*, *Pugettia quadridens*, and *Dorippe granulata* which

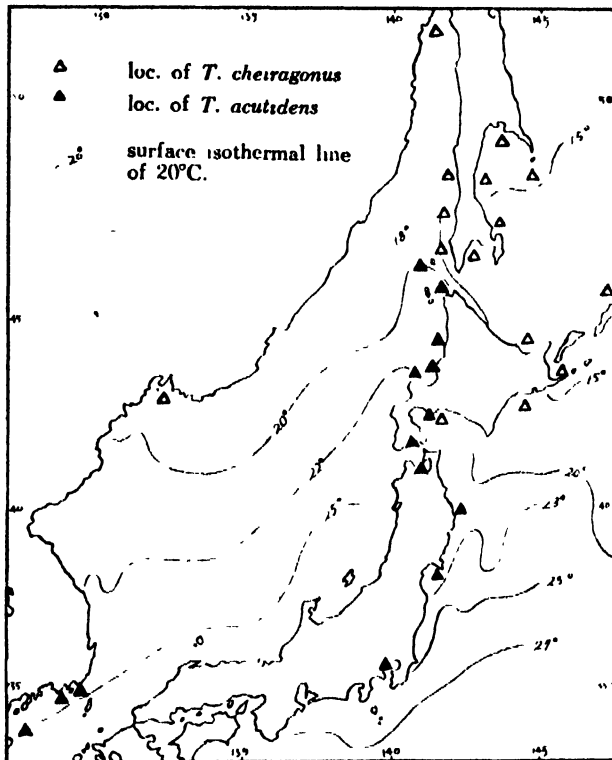


Fig. 7. Map of Japan and adjacent territories, to illustrate the relation between isothermal lines and localities of the *Telmessus* crabs in September of a normal year (Published by the Fishery Bureau, Department of Agriculture and Forestry of Japan).

inhabit a warm sea, we observe that they are found in Saghalien, confined to Kaiba Island, Aniwa Bay and the southern part of the west coast. The differences between faunae are more accurately described by BALSS (1924, p. 72) also at Vladivostok.

#### SUMMARY

1) In the study of 312 specimens of *Telmessus* crabs from Saghalien and Mutsu Bay, both *Telmessus cheiragonus* and *Telmessus acutidens* are

differentiated as distinct species, in support of the view of American carcinologists.

2) The specimen from Saghalien is *T. cheiragonus*, and that from Mutsu Bay is *T. acutidens*.

3) The growth relation between the width ( $W$ ) and the length ( $L$ ) of the carapace is as follows:

	<i>T. cheiragonus</i>	<i>T. acutidens</i>
Male	$W=1.552 L^{0.94}$	$W=1.598 L^{0.98}$
Female	$W=1.518 L^{0.94}$	$W=2.158 L^{0.84}$

4) *T. acutidens* is distinguished from *T. cheiragonus* in the following points:

	<i>T. cheiragonus</i>	<i>T. acutidens</i>
Carapace	convex, covered with granules	more convex and rougher, covered with setiform tubercles
Epibranchial spine	short and stout	longer and slenderer
Tooth at posterior base of epibranchial spine	absent	present
Incision at lateral margin of 6th abdominal segment of female	slight	strong
Periphery of female genital orifice	thickened and elevated	smooth
Southern limit of distribution in Japan and neighbourhood	Muroran, Sôya Strait, ? Vladivostok	Tôkyô Bay, ? Mutsu Bay, Chinkai Bay

5) No intermediate characters between the two species are found, except that the epibranchial spines of a specimen of *T. acutidens* larger than 67 mm. in carapace length are comparatively short, and resemble those of *T. cheiragonus* of 20–30 mm.

6) The southern limit of the distribution of *T. cheiragonus* coincides with the surface isothermal line of 20°C. in September and of 1°C. in February of a normal year, and that of *T. acutidens* with that of 25°C. in September and of 10°C. in February.

7) A limitation of biotic distribution in a shallow sea is suggested with reference to Vladivostok and the Sôya Strait.

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# THE INNERVATION OF THE CRANIAL NERVES OF THE CATFISH: *PARASILURUS ASOTUS* L.<sup>1)</sup>

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(With nine figures)

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## INTRODUCTION

The catfish (*Parasilurus asotus* L.) which belongs to the bottom feeders has the life habits living in the mud and searching for its foods chiefly by exploring the bottom with its barbels. In the adult fishes, they possess each single paired maxillary and mental barbels but in the younger stage another extra paired mental barbels are found (ATODA, '35). The body is naked and smooth, and different types of sense organs, viz., terminal buds or taste buds, small and large pit organs, are scattered over almost the whole body surface.

I undertook to study the innervation of the cranial nerves of this fish and for my work, HERRICK's paper ('01) on American catfishes was found to be very valuable and a helpful guide, and also TAKAHASHI's paper ('25) on the cranial muscular system of the same species as employed by me, is the main basis for the nomenclatures cited in this article of all nerves and muscles respectively but also from the former the general plan of presentation was followed.

In American catfishes, HERRICK ('01) has fully worked out the cranial nerves and cutaneous sense organs and other authors, WORKMAN ('00) the ophthalmic and eye muscle nerves, BROOKOVER and JACKSON ('11) the olfactory nerves and nervus terminalis by the embryological method and BERKELBACH VAN DER SPRENKEL ('15) the central relations of the cranial nerves. And also in the same species, the relation of terminal buds to its nerve fibres has been investigated by OLMSTED ('20 a, b), MAY ('25) and TORREY ('34) and of organs of latero-sensory canal, by POLLARD ('92), BUNKER ('97), and BROCKELBANK ('25), and the place of origin and method of distribution of taste buds by LANDCARE ('07), and the latero-

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<sup>1)</sup>Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 135.

sensory canals and related bones by ALLIS ('04).

At this place I wish to express my deep gratitude to Prof. S. HATAI for his continuous direction. I also wish to thank the Saitô Gratitude Foundation for the financial grant which rendered this work possible.

#### MATERIAL AND METHODS

The catfish used in this work is commonly found in muddy streams and lakes and all of the specimens were collected in the prefecture of Aomori.

In the first place, I studied the general topographical features of the chief nerve trunks by the gross anatomy of a considerable number of large catfishes.

Then the small fish of four barbels stage, about 8 cm. long, was decapitated, then the head was fixed in Bouin's solution and after decalcification imbedded in celloidin. It was cut transversely into serial section 30 micra thick and alternately stained with Delafield's haematoxylin and Heidenhain's iron-haematoxylin and counterstained with eosin and with orange G, respectively.

As it was satisfactory to trace the nerve trunks with this celloidin preparation, the following descriptions and illustrations were all based on the right side of this preparation.

As a means of control, several small fishes of six barbels stage, about 3 cm. long, were fixed in Zenker's fluid, Bouin's solution and 10% formalin, stained with Heidenhain's iron-haematoxylin and orange G. In addition to these preparations I prepared also a few series with Pal-Weigert paraffin method (SHELDON, '14).

#### DESCRIPTION OF CRANIAL NERVES

##### I. The trigemino-facial complex.

###### (a) The hyomandibular trunk.

Immediately after arising from the V+VIII ganglionic complex the hyomandibular trunk passes out laterally through its foramen in the cranial wall (Fig. 7, t.hy). As soon as the trunk emerges from the cranium, it sends off the branches for the both m. levator operculi and adductor operculi (Fig. 2, r.op). Another branch follows soon, running cephalad laterally close above the m. adductor arcus palatini and supplies it and its twig innervates the m. abductor tentaculus (Figs. 1, 4, 5, 6, r.ad.pal).

The main trunk, then extends along the inner surface of the caudal

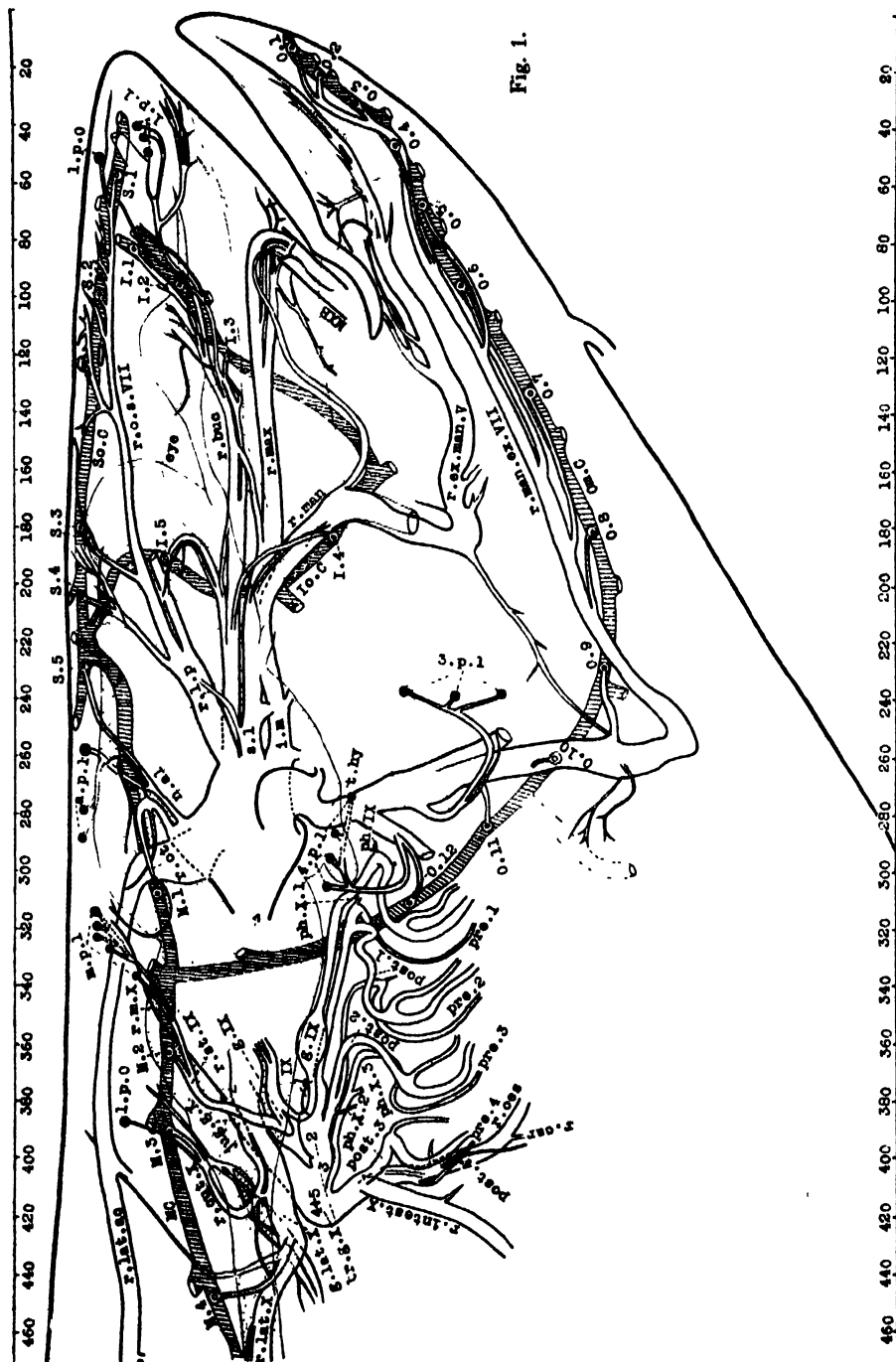




Fig. 2.

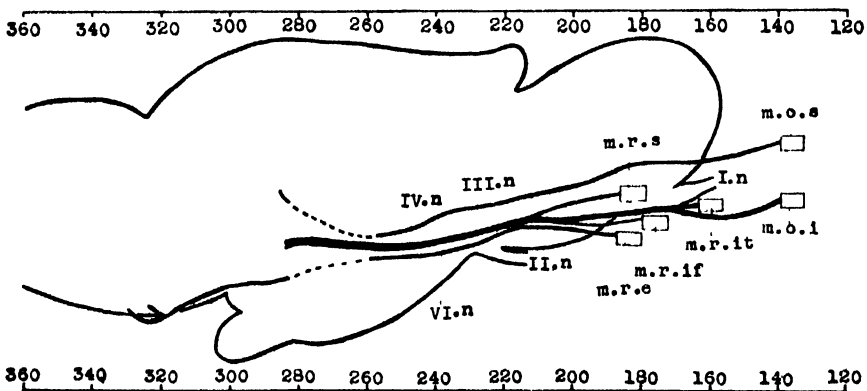
edge of the m. adductor mandibularis and divides into the r. hyoideus and r. mandibularis externus VII.

*Ramus hyoideus.* After separating from the r. mandibularis externus VII the r. hyoideus (Fig. 2, r. hy) descends into the notch between the m. adductor mandibularis and the hyomandibular bone, sending at once a small branch to the outer skin of the dorsal portion of the operculum.

The second larger branch follows soon, dividing at once into nearly equal portions of which the dorsal one runs caudad for a short distance across the ventral slip of the m. adductor mandibularis, then rises up obliquely within this muscle to the skin, supplying the two large pit organs. A slightly cephalad from these pit organs there are the other two which are presumably supplied from this same branchlet and these four organs are arranged transversely in a row at about the same level and make up the pit line (Figs. 1, 9, 4 pl). The ventral one separates dorsally from the main nerve soon after running back close to its dorsal surface and comes to lie internally of the operculo-mandibular canal, dividing into two branchlets. The larger one at once enters the pre-opercular bone therein the canal contained and supplies its last organ (12th, Fig. 1, 0.12), and the small one runs caudad under the outer skin of the operculum breaking up to supply its small pit organs.

In *Ameiurus* (HERRICK, '01) the r. hyoideus does not receive any lateralis fibres from the hyomandibular trunk and so the sense organs

Fig. 3.



Figs. 1-3. Cranial nerves of the catfish. All of the figures have been drawn from a single series of sections. The scales at the top and bottom of the figures indicate 0.6 m.m. In the figs. 1, 2, the brain and olfactory bulb are dotted, the outline of the eye is indicated by a dotted line. In the fig. 1, the lateral line canals are cross-hatched.

belonging to the acustico-lateral system in the corresponding region are all supplied by the r. mandibularis externus VII, while in my specimen it contains the lateralis fibers to some extent for the canal organ and small and large pit organs as described just above.

The main r. hyoideus, meanwhile, lies ventrally and internally of the canal and sends off four branches to the skin except the ventral most and largest one which runs back internally of the inter-opercular bone for a long distance to distribute the muscle of the operculum.

Then it descends into the branchio-stegal apparatus whose skin and muscle are innervated by it. The skin of the outer surface of the branchio-stegal membrane are rather freely supplied with terminal buds and a few number of small pit organs which may be supplied from this nerve.

*Ramus mandibularis externus facialis.* After separating from the r. hyoideus the r. mandibularis externus VII (Fig. 1, r. mand. ext. VII) passes down internally of the m. adductor mandibularis for a short distance through the notch between the preoperculum and hyomandibulare and divides into two portions. The smaller one which corresponds to the cutaneous branch of the r. mandibularis externus VII (Fig. 2, r. cut. m. ex. VII) in *Ameiurus* (HERRICK '01) descends into the most ventral angle of the m. adductor mandibularis soon after running down close above the other larger and internal branch which runs down internally of the preoperculum to the ventral side of the interoperculum.

Before the separation of these two branches a considerable branchlet leaves the nerve, running caudad obliquely within the muscle above mentioned for a short distance, then cephalad under the skin dividing into three twigs to supply the large pit organs contained in the third pit line (Figs. 1, 9, 3. pl). In other specimen, this line connects with the fourth and the second line is separable, and in another all of them connect to make up a single line extending obliquely to the mandible across the cheek, running nearly parallel with the canal of operculum and result in the long line corresponding to the cheek line of *Ameiurus* (HERRICK, '01).

The main external mandibular nerve after separating from its cutaneous branch gives off a lateral twig to the 11th organ of the operculo-mandibular canal (Fig. 1, 0.11) contained in the preopercular bone, and then as it passes down internally of the canal again sends a short twig to the 10th organ (Fig. 1, 0.10). After supplying the canal organ last mentioned two branchlets leave the nerve, of which the slightly dorsal one runs cephalad far dorsally of the main nerve and enters the preopercular bone supplying the 9th organ (Fig. 1, 0.9) of the canal and the other one runs

caudad dividing into many twigs to supply the small pit organs in the skin adjacent to the canal.

The main nerve, on the other hand, turns sharply under the interopercular bone, running forward and ventrad, then internally of this bone under the skin until it reaches the mandible. Upon entering the mandible this nerve continues to run cephalad along the inner surface of the articular bone and farther cephalad between the dentary bone and Meckel's cartilage. In the latter position it crosses externally of the r. mandibularis V and near the cephalic end enters the lateral line canal in the dentary bone to terminate its first organ (Fig. 1, 0.1).

All of the other organs of the operculo-mandibular canal are supplied with successive branches of this nerve.

The cutaneous branch, on the other hand, runs cephalad close under the skin and ventral to the most ventral angle of the m. adductor mandibularis until it comes to the ventral insertion of this muscle. At this latter point it gives off three branchlets for five large pit organs which make up the second pit line (Figs. 2, 9, 2. pl). Then it follows closely, the ventral side of the external branch of the r. mandibularis V and slightly dorsally of the main r. mandibularis externus VII for its entire length in the mandible. It gives off numerous small branchlets to the small pit organs distributed along its course and a few terminal buds and also for the skin of the caudal region of the mandible. This whole region is also plentifully supplied with terminal buds and most of them are innervated by the external branch of the r. mandibularis V.

*Ramus palatinus posterior.* In *Ameiurus*, HERRICK ('01) pointed out the abnormal position of the r. palatinus posterior, namely, the origin of this nerve was far removed from the other palatine nerve and I also recognized the same condition in my specimen.

As soon as the infero-medial strand of WRIGHT (Figs. 1, 6, i. m) separates from the ganglionic complex, the r. palatinus posterior (Fig. 2, r. pal. post) separates from its most ventral outer angle intra-cranially and passes out immediately through its foramen in the cranial wall. Then it descends at once slightly laterally into the submucosa of the roof of the mouth where it breaks up into numerous branches for the adjacent parts of the wide palate and taste buds, some small branches running out laterally, turning into the mandible and probably supply the terminal buds which are sparsely scattered over the inner surface of the skin.

*Ramus palatinus.* This nerve (Figs. 2, 4, 5, 6, r. pal) does not arise from the ganglionic complex as in the case of *Ameiurus* (HERRICK, '01).



As the both strands of Wright are still distinct extra-cranially, it separates from the ventral part of the infero-medial strand, being far cephalad of the r. palatinus posterior. It then, runs forward internally of the r. maxillaris V and dorsally of the m. adductor arcus palatini. It sends off small branches for its entire length to the submucosa of the mouth and taste buds cephalad of the region innervated by the r. palatinus posterior. Further cephalad it runs close above the premaxillary bone and supplies its teeth.

(b) The infra-orbital trunk.

This trunk comprises the r. mandibularis V, r. maxillaris V and r. buccalis. After separating from the V+VII ganglionic complex intra-cranially, supero-lateral (Figs. 1, 6, S.1) and infero-medial strands of Wright pass out through the common foramen in the cranial wall, the former lying dorsally of the latter.

Soon after separating from the ganglionic complex supero-lateral strand gives off a nerve dorso-laterally to the m. levator arcus palatini and its small twig for to m. dilator operculi (Figs. 1, 5, 6, r.l.p).

Another and larger branch follows soon, running laterally, supplying at once the m. adductor mandibularis and the remainder of the branch runs further laterally between that muscle and m. adductor arcus palatini, finally entering the m. adductor tentaculus (Figs. 1, 4, 5, r.ad.t).

*Ramus mandibularis trigemini.* As above mentioned, after emerging from the cranial foramen both strands run cephalad for a short distance, then unite (Fig. 5, s.l+i.m) to form the r. mandibularis V (Figs. 1, 2, 4, r.man) and r. maxillaris V. In this position (Fig. 4), they are arranged dorsally of the m. adductor arcus palatini, internally of the m. adductor mandibularis and ventrally of all members of the eye-muscle nerves and of the optic nerve, the former being directly external to the latter. Then the r. mandibularis V runs forward, separating laterally and externally from the r. maxillaris V until it comes under the eye and divides into two unequal branches, the external one being slightly smaller than the other, both descending at once into the mandible and turn cephalad.

The external branch (Fig. 1, r.ex.man.V), then, runs cephalad close externally of the articular bone, soon after the dentary bone for its entire length and divides into many branchlets supplying the overlying skin and its contained terminal buds, some of these branchlets running upwards and inwards to the outer edge chiefly and more inner part of the lower lip and supply them.

Farther cephalad it runs close below the dentary bone and near the

Fig. 4

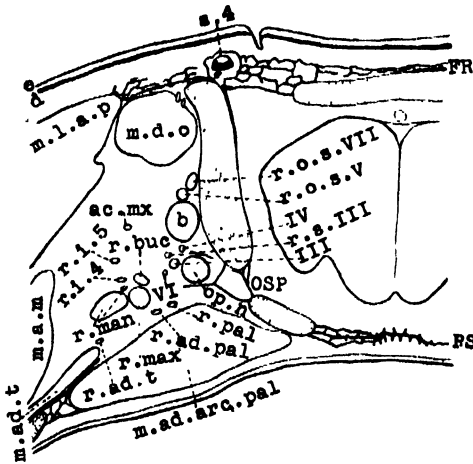


Fig. 5

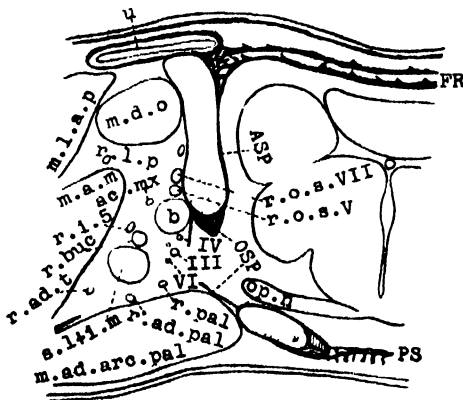


Fig. 6

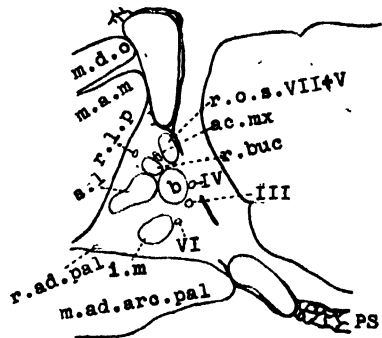


Fig. 7

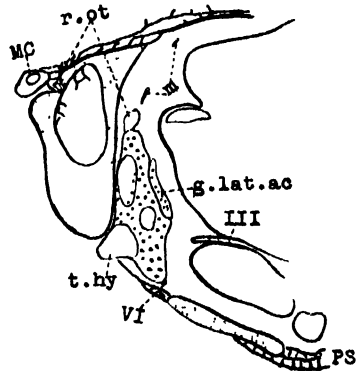


Fig. 8

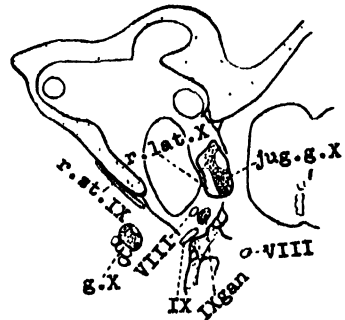


Fig. 4-8. Transections of the same preparation. The plane of the fig. 4 is 5.79 m.m. from the tip of the snout (cf. figs. 1, 2), then 6.24, 6.81, 8.16 and 11.1 m.m. ( $\times 21.8 \times 2/3$ ).

cephalic end, dividing into many branchlets and twigs to supply the teeth, though they confuse with those of the cutaneous branch of the *r. mandibularis externus VII*.

At the point where the external branch is directed cephalad in the mandible, a branchlet leaves the nerve, running back to the operculum, and supplies the terminal buds which are scattered over it. Another branchlet follows soon, running cephalad for a short distance and intimately fuses with the cutaneous branch of the *r. mandibularis externus VII* which runs directly under this external branch.

The internal branch (Fig. 2, *r. in man. V*), on the other hand, runs forward close internally of the articular bone, then, on the Meckel's cartilage and external to the ventral insertion of the *m. adductor mandibularis*. A short distance caudad of the mental barbel it gives off internally the first considerable branch which runs cephalad within the subcutaneous tissue of the lateral portion of the mandible along the inner face of the dentary bone, innervating the taste buds which are plentifully supplied along its course and its small twigs break up to the mucus lining of the lower jaw, supplying there and terminal buds.

The main internal branch, then, descends sharply along the outer surface of the cartilage to its ventral side and crosses ventrally under the *r. mandibularis externus VII* entering the mental barbel. Previously, however, it sends off a considerable branchlet to the terminal buds which are scattered over the caudal region of the mental barbel and also this branchlet presumably corresponds to the nerve that innervates the temporary mental barbels existing in the larval stage of the fish. Just before entering the barbel, it gives off a few branchlets to both *m. geniohyoideus superior* and *inferior*.

On the other hand, as soon as the *r. mandibularis V* is formed, it sends off a small branchlet to the skin under the eye and its contained terminal buds. Another and larger branchlet leaves the nerve just before turning into the mandible, running cephalad externally of the *m. adductor tentaculus* and under the eye, finally entering the maxillary barbel.

#### *Ramus maxillaris V.*

As the *r. mandibularis V* just turns into the mandible, the *r. maxillaris V* (Figs. 1, 4, *r. max*) takes up a position ventrally of the *r. buccalis* and in the narrow space between both *m. adductor* and *abductor tentaculus*, previously, however, it sends off the first considerable branch dorsally which follows the dorsal side of the main nerve for a some distance and fuses intimately with the *r. buccalis*.

The main nerve, then, runs cephalad along the m. adductor tentaculus, closely internal to it and just cephalad of the eye it divides into two unequal portions. The dorsal and larger one again divides, both soon after running forward closely accompanied with each other and enters the maxillary barbel cephalad of the nerve for this barbel which comes from the r. mandibularis V. All of the maxillary and mental barbels are exceedingly plentifully supplied with terminal buds as in the case of *Ameiurus* (LANDACRE, '07).

The ventral one follows closely below the dorsal one until the latter enters the barbel, then dividing into four branchlets of which the most ventral one runs at once caudad and the others cephalad in the lateral portion of the upper jaw, all of them supplying the lateral premaxillary teeth and the skin and taste buds in that region.

On the other hand, while the both strands of Wright are still distinct, a nerve corresponding to the accessory maxillary nerve of *Ameiurus* (HERRICK, '01) leaves them (Figs. 2, 4, 5, 6, ac.mx). It passes out between the r. ophthalmicus superficialis V+VII and r. buccalis, then runs forward laterally over the maxillary and mandibular rami and rises up to the skin from close internally of the eye. At this point, it sticks to the branch of the r. buccalis for the 4th organ of the infra-orbital canal but does not fuse, and soon divides, one directs cephalad and one caudad, both breaking up into several small twigs to supply the skin and also terminal buds in the dorsal area behind the eye.

*Ramus buccalis.* This nerve (Figs. 1, 4, 5, 6, r.buc) supplies all of the sense organs of the infra-orbital canal and three organs of the pit line (Figs. 1, 9, 1.p.l) corresponding to the organs near the anterior nasal aperture of *Ameiurus* (HERRICK, '01) and *Menidia* (HERRICK, '99).

It passes out through the common foramen in the cranial wall with both strands and lies dorsally close to the supero-lateral strand, soon sending off a branch which runs out laterally through the notch between the m. levator arcus palatini and m. rectus externus and terminates to the last organ (5th, Fig. 1, 1.5) of the infra-orbital canal, its twigs running back to supply many small pit organs of that region.

The second branch follows soon, running out further laterally but at nearly the same level as the main nerve and parallel with the r. mandibularis V, directly dorsally of it, then slightly running caudad under the eye, finally supplying the 4th organ (Fig. 1, 1.4).

The main r. buccalis runs forward internally of the supra-orbital canal and dorsally of the r. maxillaris V, receiving its branch as already de-

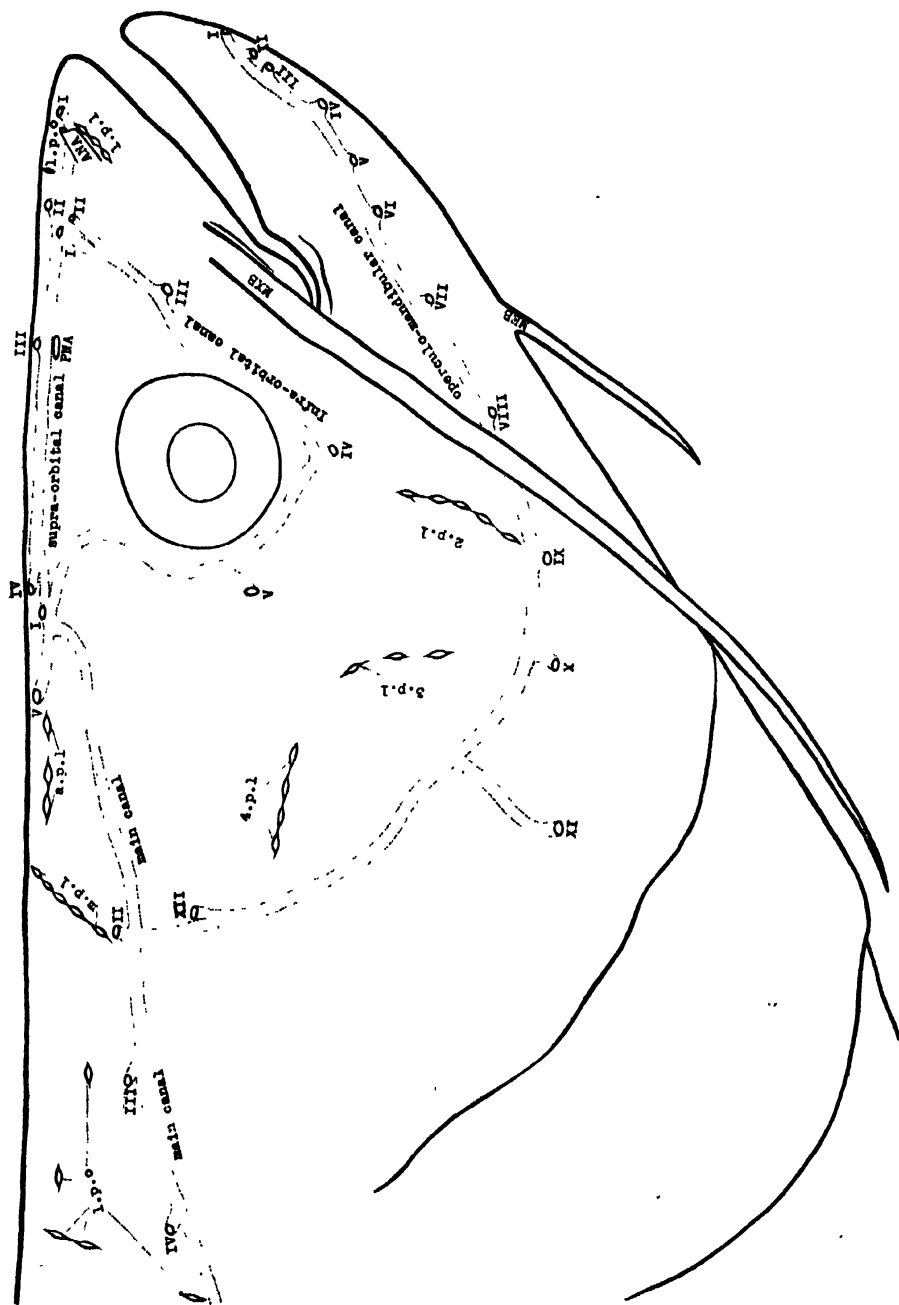


Fig. 9. Showing the pores of the lateral line canals and the large pit organs in the head of the same specimen.

scribed, sending off the successive branches for the rest of the canal organs (3rd, 2nd, 1st, Fig. 1, I. 3, I. 2, I. 1) and small pit organs which are abundant in the skin in front of the eye. Near the cephalic end, it divides into two portions, the dorsal one supplying the large pit organs above mentioned which are arranged in a row at the base of the anterior nasal aperture, and the other takes up a peculiar course. It descends along the outer side of the nasal capsule to its base, passing down through the premaxillary bone to the base of its teeth and supplies them. This may be regarded as the branch which comes from the r. maxillaris V, being previously fused with the r. buccalis.

(c) The supra-orbital trunk.

This trunk comprises the r. ophthalmicus superficialis V and r. ophthalmicus superficialis VII and the r. ophthalmicus profundus is absent in this fish as it was reported on *Ameiurus* by WORKMAN ('00).

The two ophthalmic nerves, as compared with the corresponding nerves of *Ameiurus* (WORKMAN, '00), present differences in some respects.

They combine intimately intra-cranially, the r. ophthalmicus superficialis VII being dorsal, arising from the most dorsal angle of the ganglionic complex and slightly cephalad of the origin of both strands. It runs forward for a short distance within the cranial cavity close dorsally of the supero-lateral strand and mesially and internally of the r. buccalis, then passes out through a common cranial foramen with the infra-orbital trunk in this condition. It, then, continues cephalad running close to the outer surface of the cranial wall for some distance over all members of the infra-orbital trunk and the eye-muscle nerves and under the m. dilator operculi and separates the r. ophthalmicus superficialis V from the ventral side.

In *Ameiurus*, these nerves never fuse and each has a separate foramen, not common with that of infra-orbital trunk, but their peripheral courses are almost identical and though the r. ophthalmicus superficialis V gives the appearance of the branch of the other ophthalmic nerves in my specimens, but they do not unite into the r. buccalis to form one trunk some way from the ganglion as in the case of the other siluroid, *Clarias* (POLLARD, '92).

*Ramus ophthalmicus superficialis facialis.* As above mentioned, the r. ophthalmicus superficialis VII (Figs. 1, 4, 5, r. o. s. VII) continues to run in the same direction along the outer face of the cranial wall close above the other ophthalmic nerve.

Its first branch given off soon after separating from the r. ophthal-

micus superficialis V rises up at once sharply close to the cranial wall, passing upwards across the slip of the m. dilator operculi, then perforates the frontal bone entering the 4th organ of the supra-orbital canal (Fig. 1, S. 4), its branchlet breaking up into many twigs to supply the small pit organs dorsad and caudad of the eye.

A some distance cephalad, a second and larger branch is given off which pursues a similar course to the first supplying the 3rd organ of the canal (Fig. 1, S. 3), its twig running forward under the skin and innervates the small pit organs.

The main nerve, on the other hand, continues cephalad running close to the cranial wall after sending off the 2nd branch, then raises up to the dorso-lateral angle of the supra-orbital cartilage, just internal to the m. dilator operculi and slightly ventrad of the frontal bone which contains the canal. It now comes to lie internal to the posterior nostril, just below the canal and the r. ophthalmicus superficialis V close to its ventral side and sends dorsally the short branch for the 2nd organ (Fig. 1, S. 2).

Meanwhile, before supplying the 2nd organ, the 3rd considerable and several small branches are given off dorsally, all of them breaking up into numerous branchlets to supply many small pit organs contained in the skin dorsal of the canal.

The main nerve, then, near its cephalic end, sends off several small branchlets to the small pit organs of the top of the head in the vicinity of the anterior nasal aperture, and divides into two portions, the ventral one, being slightly larger than the other, which may be regarded as the main nerve, supplying the first canal organ (Fig. 1, S. 1). The dorsal one raises up under the skin from the ventral side of the canal along its inner face to supply the small pit organs and the largest one of them turns inward near the median line of the head and terminates the paired large pit organs (Fig. 1, 1. p. 1).

*Ramus ophthalmicus superficialis trigemini.* After separating from the r. ophthalmicus superficialis VII, the r. ophthalmicus superficialis V (Figs. 2, 4, 5, r. o. s. V) follows closely to the ventral side of the former for a some distance and as the former gives off its 2nd branch, the latter begins to separate ventrad from the former. In this position, it lies slightly laterally under the m. dilator operculi and ventro-laterally of the m. levator arcus palatini.

Soon after, it sends off a considerable branch which runs out laterally and mesially and internally of the m. levator arcus palatini and raises up in the subcutaneous tissue along the outer face of the muscle last described,

after having passed through the median ethmoid dividing into two branchlets, ventral one breaking up to supply the skin and terminal buds dorsally and in front of the eye. The other branchlet runs forward under the skin along the external and dorsal face of the nasal sac, sending off many twigs for the overlying skin and its contained terminal buds.

The main nerve continues to run cephalad and ventrad over all members of infra-orbital complex and eye-muscle nerves; sending off several slender branches to the skin and terminal buds dorsally of the canal. Then it turns upwards and inwards, passing through a foramen in the dorso-lateral angle of the supra-orbital cartilage and overlying bone. It now lies close under the r. ophthalmicus superficialis VII and internal to the nasal sac and pursues in the same direction, sending off many small branchlets for the overlying skin and terminal buds, though in its termination, it interlaces freely with the other ophthalmic nerve, it supplies the skin and terminal buds dorsally and mesially of the nasal sac and the tip of the snout.

(d) Ramus oticus.

The r. oticus (Figs. 1, 7, r.ot) arises from the dorsal part of the ganglionic complex just cephalad of the origin of the r. lateralis accessorius. It runs up along the inner surface of the cranial wall for a short distance, then passes out laterally through its cranial foramen, just within which two small branches leave the nerve. It, then, turns caudad at once internally of the main canal and supplies its first organ (Fig. 1, M. 1), its branch passing through the overlying bone and breaks up to the skin adjacent to the canal. These branches probably supply the skin, terminal buds and small pit organs which are plentifully supplied in this whole region.

Just cephalad of the r. oticus, another nerve (Fig. 1, n.al) arises from the ganglionic complex. It runs up along the cranial wall intra-cranially and soon divides into two branches, one of them running cephalad for a considerable distance under the cranial roof and pierces the cranium to supply the 5th organ (Fig. 1, S. 5) of the supra-orbital canal. The other branch raises up at once to supply one of three large pit organs (Fig. 1, 9, a.p.l) extending in a row caudad from the last pore of the supra-orbital canal and make up a pit line corresponding to the anterior pit line in *Ameiurus* (HERRICK, '01). The other two organs are presumably supplied by its same branches.

Another two or three very slender nerves separate from the ganglion slightly cephalad from the nerve for the 5th organ of the canal, pierce



the cranial roof, probably supplying the skin, terminal buds and small pit organs in that region.

(e) *Ramus lateralis accessorius*.

Just caudad of the separation of the hyomandibular trunk from the ganglionic complex, a compact mass of ganglion cells swells out dorsally and internally of the ganglion, from which the *r. lateralis accessorius* (Fig. 7, g. lat. ac, Figs. 1, 2, r. lat. ac) arises. It rises up steeply to the dorso-lateral angle of the cranial cavity along the inner surface of the wall, then caudad under the cranial roof nearly to the caudal limit of the medulla oblongata before passing out through its cranial foramen. After emergence from its long cranial foramen which transversely pierces the supra-occipital bone, the nerve continues to run caudad near the median line of the body and internally then dorsally of the dorsal muscle, sending off the branches to connect with the spinal nerves.

Slightly cephalad of the origin of this nerve a very slender twig leaves the ganglion and rises up through the similar course to the *r. lateralis accessorius*, but cephalad, under the cranial roof, then pierces it, and probably supplies terminal buds in the overlying skin.

## II. The glossopharyngeal nerve.

The exit of the glossopharyngeus (Figs. 1, 8, IX) from the brain is similar to that of other Siluroids, *Ameiurus* (HERRICK, '01) and *Silurus glanis* (BERKELBACH VAN DER SPRENKEL, '15). It arises from two roots, of which the dorsal one (communis root) emerges from the brain at the nearly same level as the exit of the *r. lateralis vagi* and slightly ventrally of it, the other one (motor root) being slightly frontal and ventral from the level of the former. These two roots join together soon after running caudad directly under the root of the *r. lateralis vagi*. A short distance cephalad of the vagus foramen it passes out through the separate foramen in the cranial wall, just within which a small ganglion is formed.

From this ganglion a considerable branch (Figs. 1, 8, r. st. IX) corresponding to the *r. supra-temporalis glossopharyngei* in *Ameiurus* (HERRICK, '01) separates which rises up at once dorso-laterally along the ventral surface of the parotic process of the cranial wall, entering to the bone contained the main canal and divides into unequal branchlets. The larger one supplies at once the 2nd organ of this canal (Fig. 1, M. 2) and the other smaller one runs cephalad and breaks up to the skin along the outer surface of the dorsal muscle to supply two organs of a series of five large pit organs in a row (Figs. 1, 9, m. p. l), which correspond to those of the middle pit line of *Ameiurus* (HERRICK, '01), extending ob-

liquely and slightly caudad from the anterior pit line. The rest of the organs seem to be supplied from this same branchlet.

The main nerve, on the other hand, runs down caudad for a short distance after having passed out through its cranial foramen and turns forward, becoming ganglionic. Then it continues to run cephalad for a long distance along the ventro-lateral angle of the cranial wall until it descends down to the first branchial arch. As it enters the arch, it sends off a branch (r. pharyngeus IX, Fig. 1, r. ph. IX) which runs cephalad to the submucosa of the mouth where it breaks up into numerous branchlets supplying the taste buds in the middle part of the roof. As already described, the outer part of the palate is also freely supplied with taste buds which are innervated from the r. palatinus posterior. As *Ameiurus* (HERRICK, '01), the taste buds in the mouth and pharynx are larger in type than those of the skin.

### III. The vagus nerve.

A short distance caudad of the exit of the r. lateralis vagi from the brain the other vagus root (communis+motor) emerges from the brain, both running back in the cranial cavity, the latter root being closely internal to the former, then pass out through the vagus foramen in the cranial wall, just within which the large jugular ganglion is formed (Figs. 1, 8, jug. g. X).

The nerve arising from the most dorsal portion of this ganglion is the r. cutaneous dorsalis vagi (Fig. 1, r. cut. X) which runs caudad closely following the dorsal face of the r. lateralis vagi and as soon as the ganglion of the r. lateralis vagi is formed it receives a twig from the most cephalic and dorsal portion of that ganglion. Then it runs up around the parotic process of the cranial wall to its dorsal face, soon dividing into four branchlets, one being directed caudad and the others cephalad, all running outwards close under the dorsal muscle.

Of the latter branchlets, one runs up to the overlying skin of the main canal to supply it and the other two belong to the r. lateralis vagi, one of which at once supplies the 3rd organ (Fig. 1, M. 3) of the main canal and the other runs up to the skin to supply a single large pit organ. A branchlet directed caudad is the r. opercularis vagi and again divides into several portions under the main canal, all running down into the operculum to supply the lining of its caudal part.

A very slender nerve (Fig. 1, r. m. X), on the other hand, leaves the jugular ganglion, running up at once in the meninges around the oblongata. It, then, runs forward and upward within the cranial cavity and as the r.

lateralis accessorius emerges from the cranial cavity, it reaches the dorsal surface of the brain and further mesially of that nerve, soon passing out to the skin through the gap between the dorso-median part of the supra-occipital bones. HERRICK ('01) has described the corresponding nerves in his *Ameiurus* paper and added that "STANNIUS states that this nerve is absent in *Silurus*, but JUGE finds it present in this species". I always found it in my specimens even by the gloss anatomy.

As previously mentioned, after separating from the brain the r. lateralis vagi runs back dorsally and externally of the VIII nerve, emerging from the cranium by the vagus foramen. In the latter position (Fig. 8), it sticks closely to the outside of the jugular ganglion, then runs caudad laterally with the communis root which soon separates ventrad from the r. lateralis X, forming the large ganglion. This ganglion is quite similar to that of *Menidia*, on which HERRICK ('99 p. 109) has fully studied, and its abstract is as follows. "The vagus ganglion seems single macroscopically, but microscopically it is clearly separable into four ganglia, corresponding to the four branchial clefts innervated by this nerve. The fourth ganglion is much the largest. It includes, besides the ganglion for the nerves of the fifth gill cleft, which are much smaller than the others of the series, the ganglion for the great visceral and oesophageal rami of the vagus. The ganglia for these various rami are indistinguishably fused." And besides, in my specimen, of these four ganglia, that of the first branchial nerve is more distinctly separable than those of the others (Fig. 1, br. g. X, 4+5, 3, 2, 1).

On the other hand, as soon as the ganglion of the communis root is formed the r. lateralis X also becomes ganglionic. The first branch of those which arise from the ganglion joins at once the r. cutaneous dorsalis X supplying the 3rd organ (Fig. 1, M. 3) of the main canal and the single large pit organ. A short distance caudad, the second branch is given off from the caudal portion of the ganglion, dividing at once into nearly equal portions. The dorsal one runs upwards along the ventral face of the dorsal muscle, then cephalad under the skin, probably supplying small pit organs which are occasionally scattered over the dorsal skin of the canal. The other runs directly outward to supply the 4th organ of the canal (Fig. 1, M. 4).

#### IV. The eye-muscle nervers.

These nerves (Fig. 3) are very poorly developed like *Silurus glanis* (BERKELBACH VAN DER SPRENKEL, '15), in conformity with the life habits of the fish.

*The oculomotor nerve.* The oculomotorius (Figs. 3, 4, 5, 6, III, III. n) emerges from the brain in the cleft between the base of the midbrain and the inferior lobe (Fig. 7, III), running forward close internal to the V+VII ganglion and passes out through the cranial foramen just internal to the infero-medial strand. In this position, it lies internal to the infra-orbital trunk and ventrally of the r. ophthalmicus superficialis V+VII, then follows closely to the outer face of the optic nerve and sends off the first branch dorsally which runs out laterally and innervates the m. rectus superior (Fig. 4, r. s. III).

After giving off the first branch the main III nerve again sends off the second branch for the m. rectus inferior. It, then, runs cephalad close under the optic nerve along the external and most ventral angle of the m. rectus internus, where it divides, sending the third branch directly dorsal for this muscle and the remainder runs farther cephalad to supply the m. obliquus inferior.

*The trochlear nerve.* The trochlearis (Figs. 3, 4, 5, 6, IV, IV. n) emerges from the brain near the transition from the tectum opticum to the cerebellum. Immediately after emergence from the brain it joins the inner face of the V+VII ganglionic complex and before passing through its cranial foramen separates from the ganglion. It now lies extra-cranially and internally of the supero-lateral strand and r. buccalis across the large blood vessel and slightly dorsally of the III nerve. It continues to run cephalad laterally over the other orbital structures and directly dorsally of the optic nerve, then passes through between the m. levator arcus palatini and m. abductor tentaculus and finally innervates the m. obliquus superior.

*The abducent nerve.* The abducent (Figs. 3, 4, 5, 6, VI, VI. n) is very thin like the IV nerve, arising by two rootlets and runs cephalad just internally and ventrally of the VIII nerve. As the III nerve leaves the brain, it comes to lie at the most ventral inner edge of the geniculate ganglion, then to embed in that angle. It passes through the cranial foramen close below the inner most ventral angle of the infero-medial strand, running forward and outward under all of the other orbital structures along the ventral face of the III nerve, finally innervating the m. rectus externus.

#### SUMMARY

The topography of the cranial nerves of the catfish, *Parasilurus asotus* L. is similar to that of the American catfish, *Ameiurus melas* RAF. except

a few differences with the ophthalmic nerves and r. hyoideus on which I have already described.

The trigemino-facial complex is well developed and very intricate, while, on the contrary, the eye-muscle nerves are all poorly developed.

All of the types of sense organs reported by HERRICK ('01) on *Ameiurus* are also found in *Parasilurus*.

The terminal buds and small pit organs are freely scattered over almost the whole body surface, but much more plentiful on the head than trunk, while the large pit organs are not so plentiful on the head but are arranged transversely in many rows on the trunk.

These pit lines of the trunk were not reported by HERRICK ('01) and according to him, "the large pit organs, few in number and corresponding to the familiar pit organs of other teleosts in general and of *Amia*; the small pit organs, not commonly present in other fishes, very numerous and distributed over the whole surface of the head and trunk".

The existence of large pit organ is easily inferable macroscopically by the slit-like and whitish spot in the skin. It is not definite in number and probably in its arrangement.

The lateral line canal system in the head, like *Ameiurus* (HERRICK, '01), comprises the four, supra-orbital, infra-orbital, operculo-mandibular and main canals, the latter runs caudad into the trunk.

The pores of all canals which open to the surface are single tubes as in *Ameiurus*, *Silurus* (ALLIS, '04) and other Siluroids (POLLARD, '92) and never in a dendric manner.

The position and number of the pores are not constant, especially in

Canal	<i>Parasilurus</i>		<i>Ameiurus</i> (HERRICK)		<i>Clarias</i> (African catfish) (POLLARD)	
	No. of organ	Innervation	No. of organ	Innervation	No. of organ	Innervation
Supra-orbital	5	1st-4th..... r. o. s. VII, 5th.....n. al.	5	1st-4th..... r. o. s. VII, 5th.....n. al.	6	1st-5th..... r. o. s. 6th....same as n. al.
Infra-orbital	5	1st-5th..... r. buc.	6	1st-6th..... r. buc.	5	1st-5th..... r. buc.
Operculo-mandibular	12	1st-11th..... r. man. ex. VII, 12th....r. hy.	8	1st-8th..... r. man. ex. VII.		
Main		1st....r. ot. 2nd....r. st. IX. 3rd, 4th.... r. lat. X		Same as <i>Parasilurus</i>		1st....r. ot. 2nd....IX (r. st. IX?) 3rd....r. lat. X (r. supra-temporalis X).

those of the infra-orbital canal. In fig. 9, the sixth pore of this canal opens just before the union point of the supra- and infra-orbital canal but in others it takes up much lower position and also the second pore is removed to the position of the third and the latter to that between the second and forth.

The innervation of the canal organ of the catfish and other Siluroids are shown in the above table.

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## REFERENCE LETTERS

- ac.mx. accessory maxillary nerve.  
 ANA. anterior nasal aperture.  
 a.p.l. anterior pit line.  
 A.S.P. alisphenoid bone.  
 b. blood vessel.  
 br.g.X. 1, 2, 3, 4+5. ganglia of four branchial rami of vagus.  
 cut.ex.VIII. cutaneous branch of r. mandibularis externus VII.  
 d. dermis.  
 e. epidermis.  
 FR. frontal bone.  
 g.lat.ac. ganglion of r. lateralis accessorius.  
 g.lat.X. ganglion of r. lateralis vagi.  
 g.IX. ganglion of glossopharyngeal nerve.  
 i.m. infero-medial strand of Wright.  
 Io.C. infra-orbital canal.  
 I. 1, 1.2, . . . , I.5. organs of infra-orbital canal.  
 jug.g.X. jugular ganglion of vagus nerve.  
 l.p.o. large pit organ.  
 m. meningeal twig of r. lateralis accessorius.  
 m.ad.arc.pal. m. adductor arcus palatini.  
 m.ad.t. m. adductor tentaculus.  
 m.a.m. m. adductor mandibularis.  
 M.C. main canal.  
 m.d.o. m. dilator operculi.  
 MEB. mental barbel.  
 m.l.a.p. m. levator arcus palatini.  
 m.o.i. m. obliquus inferior.  
 m.o.s. m. obliquus superior.  
 m.p.l. median pit line.  
 m.r.e. m. rectus externus.  
 m.r.if. m. rectus inferior.  
 m.r.s. m. rectus superior.  
 MXB. maxillary barbel.  
 M. 1, M. 2, . . . , M. 4. organs of main canal.  
 n.al. nerves for anterior pit line and fifth organ of supra-orbital canal.  
 op.n. optic nerve.  
 OSP. orbito-sphenoid bone.  
 0.1, 0.2, . . . 0.12. organs of operculo-mandibular canal.  
 ph.IX. ramus pharyngeus IX.  
 ph.X. 1, 2, 3. pharyngeal rami of 1st to 3rd branchial trunk.  
 PNA. posterior nasal aperture.  
 post. 1, 2, 3, 4. post-trematic rami of the 1st to 4th branchial trunks of vagus.  
 pre. 1, 2, 3, 4. pre-trematic rami of the 1st to 4th branchial trunk of vagus.  
 PS. parasphenoid bone.  
 r.ad.pal. nerve for m. adductor arcus palatini and m. abductor tentaculus.  
 r.ad.t. nerve for m. adductor mandibularis and m. adductor tentaculus.

- r. buc. r. buccalis VII.
- r. car. ramus cardiacus vagi.
- r. cut. X. r. cutaneous dorsalis vagi.
- r. ex. man. V. external branch of r. mandibularis trigemini.
- r. hy. r. hyoideus.
- r. i. 5. branch of r. buccalis for 5th organ of infra-orbital canal.
- r. in. man. V. internal branch of r. mandibularis V.
- r. intest. X. ramus intestinalis vagi.
- r. lat. ac. r. lateralis accessorius.
- r. lat. X. r. lateralis vagi.
- r. l. p. nerve for m. levator arcus palatini and dilator operculi.
- r. mand. ex. VII. ramus mandibularis externus VII.
- r. max. r. maxillaris trigemini.
- r. oes. oesophageal rami of vagus.
- r. op. nerve for m. levator operculi and m. adductor operculi.
- r. o. s. V. r. ophthalmicus superficialis V.
- r. o. s. VII. r. ophthalmicus superficialis VII.
- r. ot. r. oticus.
- r. pal. r. palatinus.
- r. pal. post. r. palatinus posterior.
- r. s. III. branch of oculomotor nerve for m. rectus superior.
- r. st. IX. r. supra-temporalis grossopharyngei.
- s. l. supero-lateral strand of Wright.
- s. l. + i. m. supero-lateral strand + infero-medial strand of Wright.
- So. C. supra-orbital canal.
- S. 1, S. 2, . . . , S. 5. organs of supra-orbital canal.
- t. hy. hyomandibular trunk.
- u. union point of supra-orbital canal and infra-orbital canal.
- III, IV, VI, VIII, IX. oculomotor, trochlear, abducent, auditory, glossopharyngeal nerves.
- I, II, . . . , XII. pores of canals in Fig. 9.
- I. n. olfactory nerve.
- II. n. optic nerve.
- III. n. oculomotor nerve.
- IV. n. trochlear nerve.
- VI. n. abducent nerve.





# DISTRIBUTION AND SOME EXTERNAL CHARACTERISTICS OF *PHERETIMA* (PH.) *CARNOSA* (GOTO ET HATAI) FROM KOREA

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(With three figures)

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- 1899 *Perichaeta carnosa*, GOTÔ and HATAI, *Ann. Zool. Japan.*, Vol. 3, p. 15, Fig. 4.  
1900 *Amyntas hawayanus* (part), BEDDARD, *Proc. Zool. Soc. London*, p. 645.  
1900 *Pheretima carnosa*, MICHAELSEN, *Tierreich*, p. 261.  
1925 *Pheretima pingi*, STEPHENSON, *Proc. Zool. Soc. London*, pp. 891-893, (Nanking).  
1931 *Pheretima* (Ph.) *pingi*, MICHAELSEN, *Peking Nat. Hist. Bull.*, V. 2, pp. 11-13.  
1931 *Pheretima pingi*, STEPHENSON, *Proc. Zool. Soc. London*, pp. 55-56.  
1932 *Pheretima hawayana* (part), GATES, *Rec. Ind. Mus.*, XXXIV, p. 433.  
1933 *Pheretima* (Ph.) *pingi*, CHEN, *Contr. Biol. Lab. Sci. Soc. China*, IX, 6, pp. 228-234.  
1934 *Pheretima kyamikia*, KOBAYASHI, *Jour. Chosen, Nat. Hist. Soc.*, No. 19, pp. 1-4.  
1935 *Pheretima pingi*, GATES, *SMITHSON. Misc. Coll.*, Vol. 93, No. 3, p. 14.

The diagnosis of *Pheretima carnosa* given by MICHAELSEN in his *Tierreich* is as follows:

"Borstenzahlen: ca. 55/VI, VII. Erster Rückenporus auf Intsegmtf. 13/14. Gürtel vom 14-16. Segm. (=3). Männliche Poren weit von einander entfernt, ventral-lateral, zwischen ihnen 14 Borsten; Samentaschenporen 3 Paar, auf Intsegmtf. 5/6-7/8. Je 1 Paar kleine Pubertätspapillen nahe der ventralen Medianlinie vorn am 7. und 8., manchmal auch am 18. und 19. Segm., und 1 Paar hinten am 18. Segm. dicht medial an den Linien der Männlichen Poren. Dissep. 4/5-7/8 und 10/11-14/15 verdickt, 8/9 und 9/10 fehlend. 1 Paar Darmblindsäcke. Samensäcke im 11. und 12. Segm. mit dreilappigem dorsalen Rande (mittlerer Lappen: Anhangsblase?); Kopulationstaschen fehlen. Samentaschen mit länglicher Ampulle, die durch schlank keulenförmigen Divertikel, das halb so lang wie die Haupttasche ist. — L. 153, D. 7-8 mm.; Segm. 106. Japan (Tokio)."

About a half century ago, the present species was described as a new by GOTÔ and HATAI from Japan. After this, in spite of the fact that certain points are in need of revision, further notes have not been given by any one from Japan. Thus, in the view that the species has such characteristics as indicated above, some confusions arised on the systematics

of the genus. BEDDARD ('00) and GATES ('32) placed the present species in the synonymy of *Pheretima hawayana*. In 1925, STEPHENSON erected a new species, *Ph. pingi* from China (Nanking). *Ph. kyamikia* was reported by me from Korea ('34). Recently, by the good-will of Dr. OHFUCHI, a zoologist of Saitô Ho-on Kai Museum (Sendai, Japan), I had an opportunity of examining two specimens labelled *Ph. carnosa* which were collected by Mr. Koyama in Morioka, northern part of Japan. As indicated in the postscript of this paper, it is clear that it had been described by mistake, in the following points:

(1) Number and position of the spermathecal pores (and also of spermathecae). The pores are 4 pairs in 5/6-8/9 (not 3 pairs in 5/6-7/8!).

(2) Segments having the preclitellar genital papillae. Those must be revised to be VIII and IX (not VII and VIII!). Each spermathecal pore with anterior spermathecal papilla (see the section of spermathecal pores in this paper)!

(3) Position of the first dorsal pore. That must be, in most cases, in 12/13 (not in 13/14!).

(4) Presence or absence of the septum 8/9. This septum must be revised to be membranous and shoved back to the posterior side of gizzard (not absent!).

By revising these points (from the description given in this paper), *Ph. pingi* and *Ph. kyamikia* must be placed in the synonymy of *Ph. carnosa* (Goro et Hatai). Consequently, the present species is widely distributed in China, Korea, and Japan (Kyûshû, Shikoku, Central and Northern Japan, and Hokkaidô). I am inclined to believe that its occurrence in Japan is probably due to human transference from either China or Korea, or from both of them.

In the following, its distribution in Korea is described in detail.

In Keiki-dô --

Keijô, 20 clitellate and 23 a clitellate specimens, April--November.

Ryûjin (龍仁), 5 clitellate ones, August.

Heiten (餅店), 1 clitellate one, August.

Shini (振威), 2 clitellate and 5 a clitellate ones, August.

Kôka (江華), 2 clitellate ones, August.

Rensen (漣川), 3 clitellate and 29 a clitellate ones, August.

Chikusan (竹山), 1 clitellate one, August.

Risen (利川), 3 clitellate and 6 a clitellate ones, August.

Anjô (安城), 2 clitellate ones, August.

Suigen (水原), 1 clitellate one, August.

In Chûsei-hoku-dô --

Kwaisan (槐山), 2 clitellate and 1 a clitellate ones, August.

## In Chûsei-nan-dô —

Ten-an (天安), 1 clitellate one, August.

Seiyô (青陽), 1 clitellate one, August.

Reizan (禮山), 2 clitellate and 1 a clitellate ones, August.

Gasen (牙山), 1 clitellate one, August.

Zuisan (瑞山), 1 clitellate and 1 a clitellate ones, August.

## In Zenra-hoku-dô —

Kunsan (群山), 10 clitellate and 44 a clitellate ones, August.

Zenshû (全州), 1 clitellate and 30 a clitellate ones, August.

Hankyô (板橋), 1 a clitellate one, August.

## In Zenran-nan-dô —

Moppo (木浦), 3 a clitellate ones, August.

Kwantô (莞島), 1 a clitellate one, August.

Reikô (靈光), 1 clitellate and 1 a clitellate ones, August.

Junten (順天), 1 clitellate and 2 a clitellate ones, August.

## In Keishô-hoku-dô —

Bunkei (開度), 1 clitellate one, August.

## In Keishô-nan-dô —

Shinshû (晉州), 2 clitellate and 1 a clitellate ones, August.

Masan (馬山), 1 clitellate one, September.

Sansei (山清), 1 clitellate one, August.

Senseisan (千聖山), 1 clitellate one, August.

## In Kôgen-dô —

Tetsugen (鐵原), 15 clitellate and 8 a clitellate ones, August.

Kinkwa (金化), 1 clitellate and 6 a clitellate ones, August.

Sanchoku (三陟), 3 clitellate ones, August.

## In Kôkai-dô —

Angaku (安岳), 11 clitellate and 12 a clitellate ones, August.

Shariin (沙里院), 3 clitellate and 4 a clitellate ones, August.

Shinkei (新溪), 5 clitellate and 6 a clitellate ones, August.

Hakusen (白川), 2 clitellate and 4 a clitellate ones, August.

En-an (延安), 10 clitellate and 5 a clitellate ones, August.

Sainei (載寧), 1 clitellate and 3 a clitellate ones, August.

## In Heian-nan-dô —

Heijô (平壤), 5 clitellate and 48 a clitellate ones, May and August.

Chinnampo (鎮南浦), 1 clitellate and 31 a clitellate ones, August.

Chôrin (長林), 12 clitellate and 17 a clitellate ones, August.

Kaisen (价川), 2 clitellate and 4 a clitellate ones, August.

Kynjô (球場), 1 clitellate and 2 a clitellate ones, August.

Shin-anshû (新安州), 3 clitellate and 2 a clitellate ones, August.

Myôkôsan (妙香山), 1 clitellate and 22 a clitellate ones, August.

Junsen (順川), 4 clitellate and 10 a clitellate ones, August.

Môsan (孟山), 1 clitellate one, August.

## In Heian-hoku-dô —

Taiyudô (大榆洞), 2 clitellate ones, August.

Teishû (定州), 13 clitellate ones, August.

Kijô (龜城), 1 a clitellate one, August.

## In Kankyô-nan-dô —

Genzan (元山), 4 clitellate and 3 a clitellate ones, August.

Kankô (咸興), 6 clitellate and 15 a clitellate ones, August.

In Kankyô-hoku-dô —

Kisshû (吉州), 2 clitellate ones, August.

Neighbourhood of Lake Chôen (長瀾湖附近), 2 specimen, August.

A number of specimens of the present species were collected in 1933-1935 from many localities in the Korean peninsula, as indicated in the above list. Considering from this list and the localities which have been reported in China, it seems to me that probably it is widely distributed in South-Eastern Asia, including Central China, Southern Manchuria, Korea, and Japan, except the so-called Amur-region. In collections in the far east-northern part of the peninsula (also including a small part of the east-southern portion of Manchuria, Kantô (間島)), no species of the sub-genus *Pheretima* were collected, (*Lumbricid* and *Drawidian* worms are moderately abundant there). Turning to the south from there to the neighbourhood of the Lake Chôen, a few worms identical with the present species were collected together with a number of specimens of *Eisenia* sp. Here, I can say with confidence that the east-northern limit of the distribution of the sub-genus *Pheretima* and at the same time of *Pheretima* (*Ph.*) *carnosa* (GOTÔ et HATAI) in Korea is restricted to near the region of the Lake Chôen, Kankyô-hoku-dô (N. L. 41° 38'). Turning again to the south from there to Jôshin (城津), the other species of *Pheretima hupeiensis* MICHAELSEN was found, though small in number. From this research, the geographical sketch of the distribution of the sub-genus *Pheretima* figured by MICHAELSEN ('34 p. 17) must be revised, so far as concerns that in Korea. With the turning to the south from there, worms of the same sub-genus gradually increase in species and also in individuals.

In the west-northern part of the peninsula including Kôkai-dô, Heian-nan-dô, and Heian-hoku-dô, the present species is most common and most abundantly inhabited (according to the locality the other species such as *Pheretima hupeiensis* or *Ph. aggera* or *Ph. hilgendorfi* or *Ph. agrestis* or some unknown species may be rather abundant). Unfortunately, it was not found in the sandy regions of both Shingishû and Antô situated down the Ôryokkô, in spite of my collection. But, considering from the fact that several specimens were found in some localities not so far apart from these towns, this may be due to the unfavourable ecological conditions for its distribution.

It was also found together with the other species of the sub-genus from the Central Korean region, south of Keiki-dô, though the collecting

localities are small in number. An acitellate worm was collected from a small island, Kwantô (莞島), slightly apart from the south coast of the peninsula.

In the vicinity of Keijô, the worms of *Pheretima* hatched that year reach the fully matured condition after August, similarly as in *Ph. communissima* studied by HINO ('29)<sup>1)</sup> at Sendai, Japan. Considering from the following facts, it is probably a hibernated earthworm. Even in April, both fully matured and juvenile ones were collected; in November (in which time the temperature fluctuates about  $-3^{\circ}$ — $19^{\circ}$ C) it was also found as a vigorous worm in slight depth of the cultivated soil or under fallen leaves, while most of the annual worms such as *Ph. hilgendorfi* dissolve the body itself probably after the performance of the sexual function.

#### EXTERNAL CHARACTERISTICS

Two hundred and four specimens were selected for the present study, including several with incomplete clitellar glandularity. Even those latter ones were sufficient for an examination of the external characteristics, neglecting the clitellar appearance; especially with regards to the genital papillae, they were clearly recognized and not so different from those with complete clitellar glandularity. It can be said also in the present species as GATES ('31, p. 362) stated in the section of the general remarks of the genus, that the genital markings appear, at least rudimentary, long before the clitella begin to develop.

The value of variation obtained from a much number of animals collected in a single locality or very small area must be decreased, for it is always desirable that a number of specimens from widely separated localities should be examined in order to determine the degree of individual variation of the characteristics under discussion. Although the number of specimens used here is too few to fully discuss the variation of the characteristics, I believe that the following results are of a considerable significance.

In the genus *Pheretima*, body size, number of segments, first dorsal pore in situation, setal arrangement, setal number, spermathecal setae, male pore setae, and situation and occurrence of genital papillae are externally considered to be of systematic value. These were examined and noted in the table below.

<sup>1)</sup> HINO, '29, Sci. Rept. Tôhoku Imp. Univ. 4 series (Biol.), Vol. IV, No. 4.

## Annotations of TABLE I

No. = indicates number of specimen. No. 1-83 from west-northern part of the Korean peninsula. No. 84-120 from east-northern part. No. 121-192 from Central Korea (south of Keikido). No. 193-204 localities unknown.

L. indicates body length, mm.

D. indicates greatest diameter of body, mm.

S. indicates number of segments.

\* indicates specimen with incomplete clitellar glandularity.

\*\* indicates specimen broken anteriorly or posteriorly, at the time of collection.

\*\*\* indicates specimen regenerated.

§ indicates specimen somewhat poorly preserved.

III and IX indicate setal numbers on III and IX.

SVI and SVII indicate spermathecal setae on VI and VII.

SVIII/G<sub>1</sub> and SIX/G<sub>2</sub> indicate spermathecal setae and setal numbers between genital papillae (if present on both sides) on VIII (G<sub>1</sub>) and IX (G<sub>2</sub>). From these indications, not only the distance between genital papillae, but also that between spermathecal pore (or papilla) and genital papilla on each side may be inferable.

G<sub>3</sub>, G<sub>4</sub>, and G<sub>5</sub> indicate that of which the first two are genital papillae on XVIII, the former antesetal pair, the latter postsetal pair, and the last antesetal pair on XIX. po indicates that genital papillae are placed postsetally. abs indicates the absence of genital papillae. lvi (rvl) indicates that a genital papilla is placed ventrolaterally on the left side (right side) only. lmv (rmv) indicates that a genital papilla is placed midventrally on the left side (right side) only. Xvl (in No. 19) indicates that there is a single genital papilla ventrolaterally on left side only on X. 21/15, 5 (in No. 40) indicates that there are two pairs of genital papillae (antesetal) on each segment of VIII and IX; four papillae on each segment are separated from each other by equal distance (5 setae). ' indicates that there are two pairs of genital papillae which are closely situated with each other on each side.

MP indicates male pore setae.

DP indicates first dorsal pore in situation. ? 11/12 (? 12/13) indicates a well developed pore-like marking in 11/12 (12/13), but when pressure was brought to bear upon that region of the worm no fluid could not be observed exuding out through the body wall where the pore-like marking is located. & 11/12 (& 12/13) indicates a slight non-functional pore-like marking in 11/12 (12/13), (including a few cases in which such marking not situated intersegmentally but on the anteriormost margin of XII middorsally).

SP indicates the position of spermathecal papillae. an indicates that they are situated anteriorly to the intersegmental furrow; po posteriorly to the intersegmental furrow. ! indicates that there are three pairs of spermathecal papillae (pores) in 6/7-8/9.

SB indicates that the presence or absence of the setal breaks in the setal circle. both dist. indicates that both mid-dorsal and -ventral breaks are distinctly large comparing to zy and ab. d. d; v. ab. indicates that middorsal break is distinctly large but the midventral absent or very slight if present. both slight indicates that both mid-dorsal and -ventral breaks slight. d. d; v. sl. indicates that middorsal break distinctly large but the midventral slight.

" indicates the same as the above

No.	L	D	S	III	IX	SVI	SVII	SVIII/G <sub>1</sub>	SIX/G <sub>2</sub>	MP	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	DP	SP	SB
1	193	7.5	132	36	50	16	19	22/5	23/5	25	/	25	/	12/13	an	d. d; v. ab.
2	127	8	134	30	49	16	18	22/5	23/lmv	21	/	21	23	12/13	an	both dist.
3	193	8	134	32	59	19	22	26/6	26/lmv	24	/	22	/	12/13	an	"
4	184	9	113	29	54	15	18	21/4	22/abs	21	/	19	22	12/13	an	both slight
5	178	8	133	30	52	17	20	22/17	22/abs	22	22	22	/	12/13	an	d. d; v. a.
6	168	6.5	131	29	51	16	19	22/16	24/18	21	21	20	23	12/13	an	"
7	170	7.5	133	26	51	14	16	19/15	22/abs	21	20	19	18	12/13	an	both dist.
8	**	9				17	20	24/20	26/22	23	22	21	23	12/13	an	both slight

No.	L	D	S	III	IX	SVI	SVII	SVIII/G <sub>1</sub>	SIX/G <sub>2</sub>	MP	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	DP	SP	SB
9	165	8.5	120	26	48	11	12	15/abs	16/abs	15	/	/	/	11/12	po	both slight
10	168	8.5	136	28	52	15	16	21/abs	22/16	20	/	/	/	11/12	an	d. d; v. ab.
11	222	8	139	25	50	14	15	18/abs	20/15	18	/	/	/	11/12	an	"
12	***	6.5		29	54	15	16	19/abs	20/abs	18	/	18'	/	12/13	an	"
13	*		132	30	50	13	15	17/abs	19/lmv	18	/	18	/	12/13	an	"
14	* ****			32	53	16	19	21/abs	22/6	20	/	/	/	12/13	an	"
15	150	7.5	116	26	53	12	14	18/lmv	20/5	18	/	17	/	12/13	an	"
16	204	9	135	32	50	16	17	21/5	23/6	22	22	19	22	12/13	an	both dist.
17	144	8.5	138	33	52	16	20	21/16	23/18	20	/	17	22	12/13	an	"
18	148	8.5	132	31	51	16	17	19/17	22/20	23	/	19	/	11/12	an	d. d; v. ab.
19	208	9	133	30	50	13	16	19/14	20/14 Xlvi	20	/	rvi	rvi	12/13	an	"
20	174	9	125	31	60	14	16	23/4	23/5	21	9	21	/	12/13	an	"
21	* ***			30	51	13	13	19/16	20/16	20	/	lvi	/	11/12	an	both dist.
22	* ***			26	40	14	14	18/10	18/10	19	/	19	/	11/12	an	"
23	182	8.5	110	31	57	15	16	19/13	21/16	21	/	rvi	/	12/13	an	d. d; v. ab.
24	153	7	136	30	53	14	16	19/10	22/12	22	19	lvi	lvi	12/13	an	d. d; v. sl.
25	***	7		33	54	16	18	20/16	22/18	22	22	20	23	12/13	an	"
26	***	6.5		28	50	14	15	17/abs	19/15	20	/	17	21	12/13	an	"
27	150	6.5	131	28	52	14	17	19/10	22/abs	24	22	17	/	11/12	an	"
28	*		133	30	48	13	15	19/16	20/16	21	/	18	21	12/13	an	"
29	*		132	30	50	13	16	21/lvi	20/16	20	20	19	21	12/13	an	d. d; v. ab.
30	***	6.5		28	50	14	15	19/15	21/16	18	/	18	19	12/13	an	"
31	154	7	126	30	60	16	18	23/10	23/12	23	/	19	20	12/13	an	"
32	140	7	132	29	54	15	18	23/rvi	24/13	21	/	rvi	rvi	12/13	an	d. d; v. sl.
33	122	6	131	25	51	15	18	22/12	21/13	22	19	19	22	12/13	an	both dist.
34	110	6	135	29	49	13	15	18/10	21/13	19	/	16	19	12/13	an	"
35	182	8	141	31	52	14	15	18/14	21/15	23	/	/	20	12/13	an	d. d; v. sl.
36	**	8		30	52	14	15	19/10	21/12	18	lvi	17	19	12/13	an	"
37	* ***			30	50	16	17	19/5	22/7	19	/	15	20	12/13	an	both dist.
38	*		116	30	48	14	15	18/3	21/lmv	22	19	17	20	12/13	an	"
39	*	9		31	59	15	17	20/abs	22/rvi	22	/	/	/	12/13	an	"
40	*	7.5		32	50	13	16	21/15, 5	21/15, 5	21	/	18	19	12/13	an	d. d; v. ab.
41	170	7	128	32	51	14	16	19/4	20/5	19	rvi	17	/	12/13	an	"
42	202	8	133	29	54	16	15	20/15	21/15	21	/	/	/	12/13	an	both dist.
43	***	8		30	58	17	17	21/15	22/16	22	/	/	/	11/12	an	d. d; v. ab.
44	***	7		28	55	16	16	20/15	22/17	21	/	/	/	12/13	an	"
45	155	7.5	131	27	55	15	15	21/abs	23/lvi	24	/	21	/	12/13	an	"
46	***	7		31	50	14	14	17/lmv	21/4	19	/	/	/	12/13	an	"
47	*		130	29	52	16	17	17/abs	20/17	22	/	21	20	12/13	an	d. d; v. sl.
48	*		136	30	58	15	15	19/abs	23/5	20	/	/	/	12/13	an	both dist.
49	**	7.5		30	52	16	17	19/abs	21/abs	24	/	23	/	12/13	an	d. d; v. ab.
50	*		128	29	50	13	14	18/15	21/16	19	19	17	21	11/12	an	d. d; v. sl.
51	* ***			30	51	14	15	18/abs	21/19	26	/	24	/	12/13	an	d. d; v. ab.
52	186	7.5	133	25	52	16	17	20/abs	20/18	20	20	20	/	11/12	an	d. d; v. sl.
53	172	7	120	27	58	15	16	19/lvi	22/rvi	23	lvi	21	/	11/12	an	"
54	184	7	129	28	50	14	15	18/abs	20/abs	21	rvi	21	/	11/12	an	d. d; v. ab.
55	175	7.5	128	30	49	14	15	17/abs	18/abs	**	/	vi	/	11/12	an	both slight
56	***	7.5		29	53	16	18	22/20	23/20	21	/	21	/	11/12	an	d. d; v. ab.
57	***	8		29	49	16	18	22/rmv, po	21/5	20	/	/	/	11/12	an	"
58	***	7.5		33	52	16	17	20/abs	21/19	20	/	20	/	11/12	an	d. d; v. sl.
59	***	6.5		28	52	13	16	18/16	19/18	19	19	19	/	11/12	an	both slight
60	***	7		30	50	14	15	17/abs	20/5	18	/	/	/	12/13	an	d. d; v. ab.
61	***	8		31	50	16	17	20/16	20/abs	21	21	21	/	11/12	an	"
62	177	6.5	134	28	52	12	15	18/lvi	22/19	22	22	22	/	11/12	an	"
63	***	7		29	52	16	17	19/17	23/19	19	19	lvi	/	11/12	an	"
64	***	6.5		28	48	15	17	20/17	22/18	20	20	19	/	11/12	an	d. d; v. sl.
65	***	7		29	46	16	17	20/abs	21/abs	19	/	/	/	11/12	an	d. d; v. ab.
66	158	8	134	28	56	15	17	19/15	21/16	18	/	/	/	12/13	an	"
67	156	7	132	29	52	12	15	19/abs	22/14	18	18	18	/	12/13	an	"



No.	L	D	S	III	IX	SVI	SVII	SVIII/G <sub>1</sub>	SIX/G <sub>2</sub>	MP	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	DP	SP	SB
68	***	8		27	55	15	17	21/4	24/4	18	8	18	/	? 11/12	an	d. d; v. ab.
69	***	8		30	50	16	18	22/4	24/5	20	7	19	/	12/13	an	d. d; v. sl.
70	***	8		28	53	14	16	18/14	22/16	24	/	/	/	12/13	an	d. sl; v. ab.
71	***	8.5		29	55	16	17	20/abs	22/18	22	/	rvl	/	12/13	an	both slight
72	182	8	132	31	52	14	16	19/15	22/17	20	/	/	/	12/13	an	d. d; v. ab.
73	***	8.5		32	56	14	17	21/16	24/17	21	/	/	/	12/13	an	"
74	204	9	133	30	54	14	16	19/15	20/16	20	/	lvi	/	12/13	an	d. sl; v. ab.
75	214	9.5	126	32	55	14	17	21/15	24/17	20	/	/	/	12/13	an	d. d; v. ab.
76	180	9	132	29	52	16	18	22/16	23/17	20	/	/	/	12/13	an	"
77	164	7	132	29	52	16	17	20/15	24/18	21	/	/	/	12/13	an	"
78	184	8.5	132	30	51	16	18	21/abs	22/17	20	/	/	/	12/13	an	d. sl; v. ab.
79	170	7	131	29	52	16	17	20/4	22/5	20	7	18	/	12/13	an	"
80	168	7.5	129	29	53	14	17	20/abs	22/18	21	/	/	/	12/13	an	"
81	156	6.5	130	27	50	12	12	16/10, po	17/abs	18	/	18	/	& 11/12	po	d. d; v. ab.
82	*		144	28	48	12	12	15/12	18/14	12	/	/	/	& 11/12	an	"
83	*		132	31	52	14	17	20/4	20/4	18	7	17	/	12/13	an	"
84	152	7	129	31	48	15	17	18/4	20/4	21	7	20	7	12/13	an	d. d; v. sl.
85	***	7		24	48	14	16	18/3	20/4	20	8	20	/	12/13	an	"
86	149	7.5	130	27	51	14	16	20/5	22/5	20	7	20	/	12/13	an	"
87	126	7	133	26	52	16	17	20/4	21/4	19	8	19	7	12/13	an	"
88	***	7.5		27	46	10	16	16/abs	20/lvl	18	/	18	/	? 11/12	an	d. d; v. ab.
89	212	8	129	33	50	14	16	19/14	20/15	20	20	17	/	12/13	an	"
90	***	7		27	54	14	17	17/4	21/4	18	7	18	lmv	12/13	an	"
91	***	6		28	52	15	17	18/4	19/4	19	7	18	/	12/13	an	"
92			134	31	56	15	18	18/4	21/4	18	6	17	/	12/13	an	"
93	***	8.5		28	50	14	15	19/4	20/5	18	8	18	/	12/13	an	"
94	***	7.5		27	50	14	15	16/abs	18/rvl	18	/	/	/	& 11/12	an	d. d; v. sl.
95	193	7	133	27	52	14	14	15/abs	18/16	19	/	/	/	12/13	an	both slight
96	***	7.5		32	50	14	16	19/abs	19/abs	19	/	/	/	? 11/12	an	d. d; v. sl.
97	202	7	133	30	48	14	16	18/abs	18/abs	18	/	/	/	? 11/12	an	both dist.
98	***	8		32	52	14	15	17/abs	19/abs	18	/	18	/	12/13	an	d. d; v. ab.
99	* ***			25	46	14	16	16/abs	19/abs	16	/	/	/	12/13	an	"
100	210	9	136	27	47	14	15	16/abs	18/13	19	/	/	/	? 11/12	an	"
101	*		139	29	49	14	15	18/abs	19/16	19	/	/	/	? 11/12	an	d. d; v. sl.
102	*		138	30	47	14	15	18/abs	19/14	20	/	/	/	? 11/12	an	"
103	*		133	30	48	14	15	18/abs	19/abs	20	/	/	/	? 11/12	an	"
104	*		136	29	48	13	14	18/abs	19/abs	19	/	/	/	? 11/12	an	"
105	*		135	29	49	13	15	17/abs	19/abs	20	/	/	/	& 11/12	an	"
106	152	7.5	126	29	54	15	16	19/lvl, po	21/abs	17	/	rvl	/	& 11/12	an	d. d; v. sl.
107	***	8		31	52	13	14	19/abs	19/abs	17	/	/	/	? 11/12	an	"
108	215	9.5	137	28	53	15	16	17/abs	21/abs	21	/	/	/	? 11/12	an	"
109	***	7.5		30	48	13	14	16/abs	18/rvl	19	/	/	/	? 11/12	an	"
110	154	8	125	29	59	13	15	19/4	22/5	21	9	20	/	? 11/12	an	d. d; v. ab.
111	180	9	128	28	52	10	13	16/13	20/15	14	/	/	/	12/13	an	both slight
112	203	10	131	29	47	14	16	18/abs	21/abs	25	/	/	/	12/13	an	both dist.
113	***	9		32	51	15	17	18/4	21/4	20	lmv	17	6	12/13	an	d. d; v. sl.
114	242	11	139	31	53	15	16	18/abs	19/lvl	21	/	/	/	? 11/12	an	both dist.
115	180	8.5	115	30	49	15	16	18/abs	21/15	21	/	/	/	? 11/12	an	"
116	184	9	137	29	51	14	17	20/abs	20/abs	19	/	/	/	? 11/12	an	d. d; v. ab.
117	*		138	30	47	14	16	18/abs	19/14	19	/	/	/	? 11/12	an	"
118	***	7.5		31	56	14	16	21/4	22/4	19	8	19	/	12/13	an	d. d; v. sl.
119	***	6		29	52	16	16	20/3	24/4	19	8	19	/	12/13	an	d. d; v. ab.
120	145	6	115	27	52	12	16	21/lmv	21/lmv	20	/	19	/	12/13	an	"
121	184	8	132	28	51	12	15	17/abs	20/abs	16	/	15	/	12/13	an	both dist.
122	188	8.5	139	27	44	12	14	18/abs	20/lvl	17	/	/	/	12/13	an	d. d; v. ab.
123	177	8	138	25	44	12	16	16/abs	17/abs	14	/	/	/	12/13	an	"
124	157	7	117	25	46	11	14	16/abs	19/4	18	rmv	16	/	12/13	an	d. d; v. sl.
125	***			26	46	12	15	17/abs	19/4	18	/	18	/	& 11/12	an	d. d; v. ab.
126	150	8.5	114	26	44	12	13	16/9, po	17/abs	16	/	/	/	12/13	po	"

No.	L	D	S	III	IX	SVI	SVII	SVIII/G <sub>1</sub>	SIX/G <sub>2</sub>	MP	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	DP	SP	SB
127	179	8.5	135	24	49	10	12	16/9, po	18/abs	16	/	/	/	12/13	po	d. d; v. ab.
128	204	8.5	132	30	50	12	14	16/abs	20/abs	14	/	/	/	12/13	po	"
129	133	7	123	23	52	11	12	16/abs	18/abs	15	/	/	/	12/13	po	both slight
130	**	6.5		33	53	12	15	20/4	22/5	18	lmv	20	/	12/13	an	d. d; v. ab.
131	179	7.5	131	26	44	10	12	14/abs	16/abs	14	/	/	/	? 11/12	an	both dist.
132	***	8		24	47	11	12	15/abs	17/abs	13	/	/	/	& 11/12	an	"
133	205	7.5	134	26	42	10	12	14/abs	15/abs	14	/	/	/	? 11/12	an	"
134	***	8		31	51	14	15	19/abs	20/abs	18	/	/	/	? 11/12	an	"
135	176	7	135	26	46	11	12	14/abs	16/abs	13	/	/	/	& 11/12	an	"
136	***	7		24	44	10	11	14/abs	16/abs	12	/	/	/	? 11/12	an	"
137	***	7.5		27	47	10	11	14/10	16/13	12	/	/	/	& 11/12	an	"
138	183	9	137	24	47	11	13	14/8	16/abs	14	/	/	/	& 11/12	an	"
139	200	7.5	134	24	43	10	11	14/abs	15/abs	12	/	/	/	& 11/12	po	"
140	191	7.5	136	25	42	11	12	16/abs	16/abs	14	/	/	/	? 11/12	po	"
141	***	7		24	50	10	11	15/abs	16/abs	15	/	/	/	& 11/12	an	"
142	**	7.5		29	45	12	14	16/abs	17/abs	17	/	rvl	/	& 11/12	an	"
143	**	7.5	132	58				22/4	25/4	21	/	19	/	12/13	an	d. d; v. ab.
144	*		134	32	50	11	14	17/abs	22/abs	18	/	/	/	12/13	an	"
145	*		126	29	44	11	13	16/abs	17/abs	16	/	/	/	12/13	an	"
146	*		136	28	44	12	14	16/abs	16/abs	17	/	/	/	& 11/12	an	"
147	* ***			32	52	14	15	18/4	22/4	20	/	18	/	12/13	an	"
148	*		127	28	48	12	13	14/abs	16/2	16	rmv	16	/	12/13	an	"
149	184	8	121	26	46	10	12	15/abs	16/abs	12	/	/	/	12/13	an	"
150	***	7		27	50	13	14	17/10, po	19/abs	14	/	14	/	? 11/12	po	both dist.
151	137	6	129	26	45	13	14	19/abs	19/abs	16	/	/	/	12/13	an	"
152	**	7		36	63	16	19	23/4	24/4	20	/	20	/	12/13	an	d. d; v. ab.
153	172	8	146	30	47	11	14	16/10, po	18/abs	19	/	19	/	? 11/12	po	"
154	**	7		30	49	14	15	20/4	21/4	20	/	20	/	12/13	an	d. d; v. sl.
155	164	8	121	25	42	11	14	16/abs	16/13	14	/	/	/	12/13	an	d. d; v. ab.
156	**	10.5		24	47	10	12	16/10, po	17/abs	14	/	/	/	12/13	po	d. d; v. sl.
157	160	8.5	128	28	50	14	15	16/abs	20/abs	20	8	19	/	? 11/12	an	both dist.
158	189	9	140	31	59	15	17	19/abs	22/17	19	/	/	/	? 11/12	an	"
159	* ***			27	53	11	11	15/abs	16/abs	15	/	/	/	? 11/12	po	"
160	162	8.5	125	30	48	12	12	13/11	17/13	14	/	/	/	12/13	an	d. d; v. ab.
161	198	8	136	30	58	14	16	21/abs	22/18	22	/	/	/	12/13	an	"
162	***	7		28	52	14	15	19/abs	21/abs	20	/	/	/	12/13	an	"
163	223	8	122	30	46	11	13	17/12, po	19/13	20	/	/	/	? 11/12	an	both dist.
164	247	9	140	33	49	14	15	18/14, po	19/15	17	/	/	/	? 11/12	an	d. d; v. ab.
165	174	7	120	30	52	14	16	20/15, po	21/4	18	4	17	/	12/13	an	"
166	214	8	139	31	54	12	15	18/lvl	20/17	19	/	/	/	? 11/12	an	both dist.
167	***	7.5		29	48	14	16	19/abs	20/15	19	/	18	/	& 11/12	an	d. d; v. ab.
168	195	9.5	138	29	53	12	14	20/16	22/18	20	/	/	/	? 11/12	an	both dist.
169	199	7.5	131	33	50	14	16	17/rvl	22/lvl	20	/	/	/	? 11/12	an	d. d; v. ab.
170	***			31	52	12	14	16/lvl, po	18/4	18	lmv	15	/	12/13	an	both dist.
171	***			31	50	14	15	17/abs	19/abs	18	/	/	/	12/13	an	"
172	176	6.5	118	27	48	12	14	16/4	17/5	20	/	20	/	12/13	an	d. d; v. ab.
173	173	7.5	134	32	49	15	16	19/abs	22/abs	19	/	19	/	12/13	an	"
174	**	7.5		28	50	12	14	16/14	18/16	17	/	/	/	12/13	an	"
175	**	6.5		32	54	15	16	19/abs	20/abs	18	/	16	/	? 11/12	an	"
176	166	8.5	113	29	51	14	16	18/abs	18/13	16	/	/	/	? 11/12	an	d. d; v. sl.
177	148	9	120	30	52	13	15	18/14, po	22/abs	18	/	18	/	12/13	an	d. d; v. ab.
178	195	8.5	133	30	46	12	13	14/abs	16/2	16	/	16	/	12/13	an	"
179	193	7.5	139	27	51	11	12	16/13	18/13	14	/	/	/	& 11/12	an	"
180	158	7	122	25	46	13	12	15/abs	17/lmv	15	/	/	/	? 11/12	an	"
181	*		135	28	49	14	16	18/abs	19/abs	19	/	/	/	? 11/12	an	d. d; v. sl.
182	195	7.5	134	27	48	12	12	16/12; lvl, po	18/13	14	/	/	/	& 11/12	an	d. d; v. ab.
183	**	8	130	51	13	16		22/4	23/4	20	6	18	/	12/13	an	"
184	***	6.5		29	51	14	16	20/14, po	20/4	16	/	16	/	& 11/12	an	"
185	155	7	129	24	50	12	13	17/13, po	20/4	15	/	15	/	& 11/12	an	"

No.	L	D	S	III	IX	SVI	SVII	SVIII/G <sub>1</sub>	SIX/G <sub>2</sub>	MP	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	DP	SP	SB
186	*		127	30	51	14	15	18/abs	19/abs	16	/	16	/	? 11/12	an	d. d; v. ab.
187	205	8	126	26	50	12	13	16/abs	19/abs	16	/	/	/	12/13	an	d. d; v. sl.
188	* ***			27	51	13	14	17/abs	20/abs	16	/	/	/	12/13	an	d. d; v. ab.
189	*		134	29	48	14	14	16/abs	17/abs	17	/	/	/	12/13	an	"
190	**	7		24	50	11	14	16/12	17/12	14	/	/	/	? 11/12	an	"
191	***	6.5		29	57	14	16	20/14, po	23/16	19	/	lvl	/	? 11/12	an	both dist.
192	§		136	25	60	10	11	17/9, po	18/abs	15	/	/	/	12/13	po	d. d; v. sl.
193	***	7.5		32	52	16	16	19/4	22/6	20	9	18	/	12/13	an	d. d; v. ab.
194	* ***			27	54	11	12	14/abs	16/abs	15	/	/	/	12/13	an	"
195	168	7	130	27	45	11	12	17/4	16/4	18	6	18	/	12/13	an	both dist.
196	**	8.5		25	46	12	14	18/abs	17/abs	17	/	/	/	▲ 11/12	an	d. d; v. sl.
197	§			28	50	10	12	16/12, po	17/abs	15	/	/	/	12/13	po	d. d; v. ab.
198	§			29	49	11	12	16/abs	19/abs	15	/	/	/	12/13	an	"
199	178	8.5	134	24	47	12	13	16/9, po	17/abs	14	/	/	/	▲ 11/12	an	"
200	202	9	123	26	42	12	14	16/abs	18/rvl	17	/	/	/	? 11/12	an	both dist.
201	203	8.5	139	31	49	14	15	16/abs	21/16	18	/	/	/	11/12	an	d. d; v. sl.
202	192	9	121	28	52	14	15	18/abs	20/rvl	20	/	/	/	▲ 11/12	an	d. d; v. ab.
203	232	9	140	31	50	14	15	19/abs	20/16	18	/	/	/	? 11/12	an	d. d; v. sl.
204	§		139		44	13	14	16/abs	17/abs	16	/	/	/	12/13	an	d. d; v. ab.

## (1) Body size :

Body length of the worms varies from 110-247 mm. (in 103 specimens), and the majority are found to range between 150-210 mm.; the average length is 178 mm. The largest diameter of the worms varies from 6-11 mm. The largest specimen measured 247×9 mm. (or 242×11 mm.); the smallest, 110×6 mm. So far as both length and diameter are concerned some variations due to technique are unavoidable, but when the body size is taken, the approximate size may be calculated. According to CHEN ('33) and STEPHENSON ('31), the body size of the Chinese form is 160-340 mm. in length and 6-10 mm. in diameter.

## (2) Number of segments :

Number of segments varies between 110-146 (in 130 specimens). The frequency of occurrence in each number are indicated below. From it, the number of segments may be said to be nearly uniform to the species within the range of about 128-139. According to CHEN, in the Chinese form it varies between 110-179. It can be recognized that there is a large gap between both forms in the largest number of segments, and at the same time it is presumable that there may be also a large gap between them in the mode of number of segments.

## (3) Multilation of the body :

As shown in Table I, a number of specimens had been mutilated before they were collected. It was of much interest to me to find that nearly every worm collected at a single area, such as in Chôrin, had been mutilated before they were collected. Similar observation was reported

TABLE II

No. of segments	No. of specimens	No. of segments	No. of specimens
110 .....	1	129 .....	6
111 .....	0	130 .....	5
112 .....	0	131 .....	8
113 .....	2	132 .....	14
114 .....	1	133 .....	12
115 .....	2	134 .....	12
116 .....	2	135 .....	6
117 .....	1	136 .....	9
118 .....	1	137 .....	3
119 .....	0	138 .....	5
120 .....	4	139 .....	8
121 .....	3	140 .....	3
122 .....	2	141 .....	1
123 .....	2	142 .....	0
124 .....	0	143 .....	0
125 .....	3	144 .....	1
126 .....	5	145 .....	0
127 .....	2	146 .....	1
128 .....	5		
		Total 130 specimens	

by GATES ('32, p. 378). In mutilated worms, he also stated, the wound may heal over without regeneration, or the lost portion may be regenerated. Except some cases, whether it is normal or regenerated was clearly distinguished in the general appearance of the worm. But, such exception must be always expected in the study of this kind, so far as whether or not the number of segments regenerated is equivalent to the number lost remains to be demonstrated. But, if the specimens used are apparently normal and such doubtful ones are excluded, the extent of the variation in the body size and also in number of segments may be recognized to be of significance, and may fall within certain limits as also the same author hinted. Comparing both Chinese and Korean forms on this view point, the difference of the degree of variation in number of segments between them seems to me to be too large. Otherwise, some of the specimens I examined may be reduced to what they had mutilated before were collected.

#### (4) First dorsal pore:

The position of the first dorsal pore is an important specific characteristic; and its variation, according to species, may not be found at all, or may be considerable. In the present species, both authors STEPHENSON and CHEN simply described, not touching on the variability of this organ, that the first dorsal pore is situated in the intersegmental furrow 12/13. In the Korean form, it is considerably variable.

# Four cases observed :

(a) Definitely functional first dorsal pore is situated in the intersegmental furrow 12/13.

(b) Similar pore in the intersegmental furrow 11/12.

(c) A well developed (as similarly as the functional pore) but non-functional one in the intersegmental furrow 11/12 or 12/13.

(d) Non-functional, slight pore-like marking in 11/12 or 12/13 ; here also are included several cases in which not only either the marking in 11/12 or 12/13 or both of them are non-functional, but also sometimes that in 13/14 or seldom that in 16/17 are non-functional ; and including a few cases in which they have a non-functional, slight pore-like marking not located in the intersegmental furrow but on the anteriormost margin of XII mid-dorsally. The results are as follows :

( a ) . . . . .	115 specimens
( b ) . . . . .	2
( c ) . . . . .	57 (11/12) ; 3 (12/13)
( d ) . . . . .	27
<hr/>	
Total	204 specimens

# (5) Setae :

## (a) Setal break :

Presence or absence of setal break in the setal circle and their intervals are of a specific characteristic. After STEPHENSON, it was described as follows : "dorsal break somewhat irregular, small, usually equal to about  $1\frac{1}{2}$  yz ; ventral break indistinguishable behind clitellum, small and irregular in front", and in CHEN's description "both dorsal and ventral breaks slight, aa=1.2-2.0 ab, zz=1.2-1.5 yz". Not only according to individuals, but also partly in the same specimen, they are variable. In the present study, both intervals, mid-dorsal and -ventral were not measured by the general rule, but apparently classified into the following four groups.

1. Both mid-dorsal and -ventral breaks distinctly large . . . . .	45 specimens
2. Mid-dorsal distinctly large but mid-ventral indistinguishable or very slight if present . . . . .	106
3. Mid-dorsal distinctly large and mid-ventral slight . . . . .	44
4. Both mid-dorsal and -ventral slight . . . . .	49
<hr/>	
Total	204 specimens

This classification and the results are subject to be rough, but the tendency of variability of this characteristics may be recognized. The letter used in the above table "distinctly large" means that the setal interval aa or zz is larger than ab or zy. As is clear in this results, the

mid-dorsal break is usually present, but the mid-ventral break is rather variable and is generally slight or indistinguishable.

(b) Setal numbers on III and IX.

Setae on segments of II-IX are usually enlarged, especially those of the ventral side. Setal numbers were counted conveniently on III and IX, for the work can be more easily carried on in such segments than in the other segments having smaller setae.

On III, it varies between 23-36. In the Chinese form it varies between 24-29. In the present study, the majority were found to range between 27-31, as indicated in the table below. Considering from these circumstances, it may be recognized that there is also a slight difference between both forms. On IX, it varies between 40-63. These details are indicated below.

TABLE III (on III)

No. setae	No. specimens	No. setae	No. specimens
23 .....	1	30 .....	36
24 .....	11	31 .....	22
25 .....	11	32 .....	15
26 .....	15	33 .....	7
27 .....	21	34 .....	0
28 .....	25	35 .....	0
29 .....	34	36 .....	2
		Total	200 specimens

TABLE IV (on IX)

No. setae	No. specimens	No. setae	No. specimens
40 .....	1	52 .....	35
41 .....	0	53 .....	13
42 .....	4	54 .....	11
43 .....	1	55 .....	5
44 .....	8	56 .....	4
45 .....	3	57 .....	2
46 .....	11	58 .....	5
47 .....	9	59 .....	4
48 .....	17	60 .....	3
49 .....	14	61 .....	0
50 .....	34	62 .....	0
51 .....	19	63 .....	1
		Total	201 specimens

(c) Spermathecal setae :

Spermathecal setae were counted on VI-VIII by the usual method.

Those on IX shown in Table I, was attempted for the purpose of indication of correlation of both distances, between spermathecal lines and between genital papillae if present on that segment. On VI, it varies between 10-19; on VII, 11-22; on VIII, 13-26. Details are indicated below.

TABLE V

No. setae on VI	No. specimens	No. setae on VII	No. specimens	No. setae on VIII	No. specimens
10 .....	13	11 .....	6	13 .....	1
11 .....	18	12 .....	20	14 .....	10
12 .....	29	13 .....	11	15 .....	8
13 .....	21	14 .....	28	16 .....	35
14 .....	63	15 .....	43	17 .....	21
15 .....	24	16 .....	45	18 .....	36
16 .....	31	17 .....	30	19 .....	36
17 .....	3	18 .....	12	20 .....	24
18 .....	0	29 .....	4	21 .....	16
19 .....	1	20 .....	3	22 .....	11
		21 .....	0	23 .....	4
		22 .....	1	24 .....	1
				25 .....	0
				26 .....	1
Total 203 specimens		Total 203		Total 204	

Comparing these to those of the Chinese form, it may be said that the spermathecal setae of the Korean form are larger in number than the latter.

(d) Male pore setae:

Male pore setae vary between 12-26. In the Chinese form it varies between 12-20. Besides, as shown in the following table the usual number of the Korean form may be said to be from 18-21. It seems to me that this difference between both forms is also considerable.

TABLE VI

No. setae	No. specimens	No. setae	No. specimens
12 .....	5	20 .....	39
13 .....	2	21 .....	23
14 .....	15	22 .....	12
15 .....	10	23 .....	5
16 .....	14	24 .....	5
17 .....	10	25 .....	2
18 .....	32	26 .....	1
19 .....	28		
		Total 203 specimens	

(e) Local variation of the spermathecal and male pore setae :

Considering from the distribution of the present species, the Korean peninsula may be subdivided into the following three regions, namely : (1) west-northern region including Kôkai-dô, Heian-nan-dô, and Heian-hoku-dô ; (2) east-northern region including Kôgen-dô, Kankyô-nan-dô, and Kankyô-hoku-dô ; (3) Central Korea including the south of Keiki-dô, except Quelpart Island.

Summarizing the spermathecal and male pore setae according to these three regions, the results are as follows :

TABLE VII

	West-northern	E. N.	C. K.
Spermathecal setae on VI	11-19	10-16	10-16
on VII	12-22	13-18	11-19
on VIII	15-26	15-21	14-23
Male pore setae	12-26	14-25	12-22

From this table, the differentiation of these setal numbers in each region may not be clearly found. But, from the next table, the specimens belonging to the west-northern region may be said to form a group which has the distinctly increased number of setae compared to the other

TABLE VIII

*Number of specimens having more than 20 setae in spermathecal (on VIII only) and male pore setae*

	W. N.	E. N.	C. K.
Spermathecal setae on VIII	44 (in 83) (53.0%)	6 (in 37) (16.2%)	10 (in 72) (13.9%)
Male pore setae	59 (in 83) (71.1%)	14 (in 37) (37.8%)	12 (in 72) (16.7%)

regions ; and the Central Korean specimens — a group which has the distinctly small number of setae ; and the east-northern specimens — a group which has the intermediate number of setae. So, only with regards to the setal number, the Central Korean specimens are rather closely related to the Chinese form.

(6) Spermathecal pores and spermathecal papillae :

Spermathecal pores are four pairs in 5/6-8/9. Each pore is rather



large, but sometimes may appear to be small, and are situated on the posterior side of a transversely oval or round papilla which is flat-topped or slightly depressed centrally, distinctly raised as large as the other genital papillae, usually close anteriorly to the intersegmental furrow, but seldom posteriorly to the intersegmental furrow. Here, I propose to term such papillae "Spermathecal papillae". Very seldom, the spermathecal pores and at the same time spermathecal papillae are three pairs; such case was found in two specimens, and both in 6/7-8/9 reducing the first pair.

Spermathecal papillae placed anteriorly to the intersegmental furrow 190

Spermathecal papillae placed posteriorly to the intersegmental furrow . . 14

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Total 204 specimens

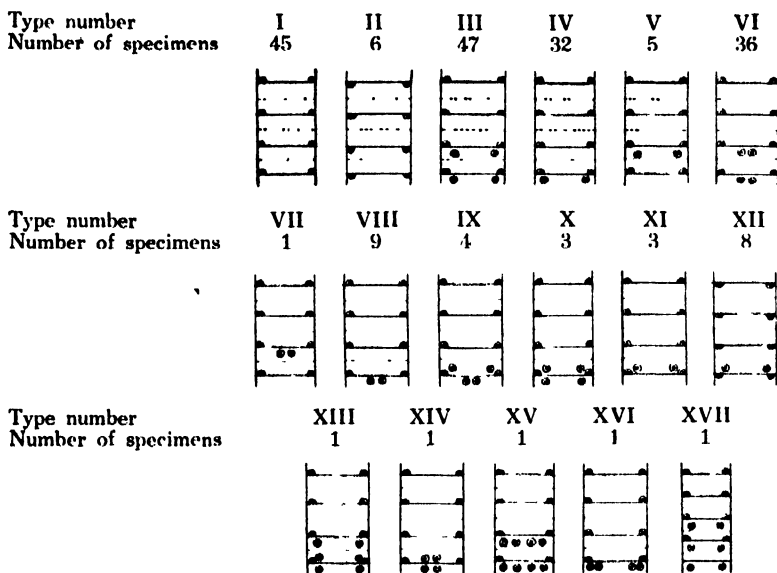
#### (7) Genital papillae:

Genital papillae can be classified into two groups corresponding to their positions anterior and posterior to the clitellum. While I was engaged in the study of the variation of both genital papillae preclitellar and postclitellar, an idea much attracted me that the frequency and position either ventrolateral or midventral in occurrence of these markings on both regions of the body may not simply happen at random respectively, but there may be something producing these on both regions coordinately or at least correlatively, as CHEN ('31, STEPHENSON, p. 56) already hinted in his letter to STEPHENSON. But, unfortunately, I cannot here clearly demonstrate the fact for such an idea, since I have not a sufficient number of specimens at hand.

##### (a) Preclitellar genital papillae:

Preclitellar genital papillae are usually situated antesetally on VIII and IX and either ventrolaterally (being slightly medial to the spermathecal papillae) or midventrally. Not only the variation in occurrence of these markings are large, but, strictly saying, their actual situations are also considerably variable, such as being situated very close to the intersegmental furrows or rather close to the setal line or situated in various intervals between themselves on each segment. In spite of such variation there are two cases, i. e., papillae placed (1) ventrolaterally and (2) midventrally, were clearly distinguished from each other. This discrimination and also the various intervals may be recognized referring the difference of the setal number in SVIII/G<sub>1</sub> and SIX/G<sub>2</sub> (in Table I). In determining the variation, taking the cases in which a single papilla occurred either on the right side or left side only into consideration, the result is rather confusing, so in the following schematic figures such unpaired ones were

all illustrated as paired ones. Thus, seventeen cases were determined. As shown, the specimens having no genital papillae on any preclitellar segments numbered 51 (in 204). This is, no doubt, a great reduction of the preclitellar genital papillae. There were 47 cases in which the ventro-lateral pairs were found on both segments VIII and IX as in the type.



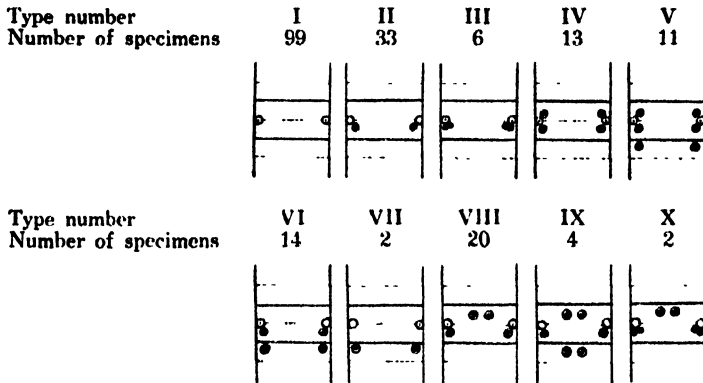
Text-fig. 1. Seventeen types of the preclitellar genital papillae.

At first sight, such a number may appear to be too small. But, including its reduced forms, such as the types (IV) and (V), the number rises to 84 cases. Those placed midventrally (similarly including its reduced forms) are 46 cases. Generally, the frequency of multiplication of them is very small. The reduction of them tends to occur more frequently on VIII than on IX. On the contrary, the multiplication of them, though with only a few cases at hand, appears to occur posteriorly to IX, not anteriorly to VIII. These papillae, sometimes, are situated postsetally on VIII if one pair, and both antesetally and postsetally if two pairs. But, no cases were found, in which the postsetal pair was situated on IX as described by MICHAELSEN ('31), (his description is as follows: once a pair of postsetal on VIII or IX). Specimens belonging to the types (II) and (XII) have spermathecal papillae posteriorly placed to the intersegmental furrows. It was much interested to me that in more than half of such specimens (14), though too few in number, both papillae spermathecal and genital

are identical in situation being the former placed "posteriorly" to the intersegmental furrows and the latter placed "postsetally", and more, that the remaining all have no genital papillae.

(b) Postclitellar genital papillae:

Postclitellar genital papillae are also variable in occurrence, but less than the preclitellar. Determining by the similar method used in the case of the preclitellar, the variations were classified into the following ten types. As is clear in it, the number of specimens having no genital



Text-fig. 2. Ten types of the postclitellar genital papillae.

papillae near the male pore region numbered 99, and that number forms the majority of all cases classified here and is nearly half of all specimens examined. On the Chinese form, CHEN gave the following description: "Genital papillae rarely absent, one to three pairs (occasionally five pairs) usually placed around the male pore region". Considering from this difference between both forms Chinese and Korean, the great reduction of the postclitellar genital papillae is apparently a distinct characteristic for the latter form. Those as in the type (after the same author ('31, STEPHENSON, p. 56) in the Chinese form such a case is rare and usually more pairs are found), i. e. postsetal pair situated slightly medial to the male porophore on XVIII are only 40 cases, though they were usually found, so far as the postclitellar papillae occurred. Sometimes, those placed on such situation are abnormally of two pairs, and in this case two and male porophore on one side are closely crowded like a mass.

Antesetal papillae occurred on two portions, one on XVIII and the other on XIX. Specimens having all three pairs of them are small in number, and no one specimens having more than three pairs of them

was found, except the abnormal cases above described. Antesetal pairs are classified again into two cases according to their situations, one is the ventrolateral and the other is the midventral, as similarly as those in the preclitellar, though in the latter case the distance between them

TABLE IX

*Showing the number of specimens having or not having the genital papillae on both regions preclitellar and postclitellar ; and those in local variation*

Precl. Postcl.		abs (I & II)				Ventrolateral (III, IV & V)				Mid-ventral (V, VII & VIII)				Abnormal (IX-XVII)				Total sum
		N.	N.	C.	U	N.	N.	C.	U	N.	N.	C.	U	N.	N.	C.	U	
		W.	E.	K.	n.	W.	E.	K.	n.	W.	E.	K.	n.	W.	E.	K.	n.	
abs (I)	N. W.	2				16				4				1				23
	N. E.		10				9				0				1			20
	C. K.			24				14				1				7		46
	Unk.				4				4				0				2	10
Total		40				43				5				11				99
Ventro- lateral (IV, V, VI & VII)	N. W.	1				30				6				2				39
	N. E.		0				1				0				0			1
	C. K.			0				0				0				0		0
	Unk.				0				0				0				0	0
Total		1				31				6				2				40
Mid- ventral (VIII, IX & X)	N. W.	0				0				5				0				5
	N. E.		0				0				12				0			12
	C. K.			1				0				4				2		7
	Unk.				0				0				2				0	2
Total		1				0				23				2				26
Post- setal (II & III)	N. W.	3				8				4				1				16
	N. E.		1				1				1				2			5
	C. K.			5				1				7				5		18
	Unk.				0				0				0				0	0
Total		9				10				12				8				39
Total sum		6	11	30	4	54	11	15	4	19	13	12	2	4	3	14	2	204

Precl ..... Preclitellar genital papillae.  
 Postcl ..... Postclitellar genital papillae.  
 abs ..... No papillae.  
 N. W. .... West-northern region.  
 N. E. .... East-northern region.  
 C. K. .... Central Korean region.  
 Unk (or Un) .. Localities unknown.

on each segment is slightly wider, as can be calculated from the difference of the setal number in Table I. The number of specimens of the ventrolateral case are 40, and that of the midventral case are 26.

As already hinted at the beginning of this section, it seems to me that there may be something producing the coordinate or at least correlative frequency and position in occurrence of genital papillae on both regions preclitellar and postclitellar. The results indicated in Table IX may be said to be some instances seeming to be suitable to explain such an idea. In the second and third columns, we see that each case of the ventrolateral and midventral papillae is coordinate in position, i. e. in the former case 31 specimens are ventrolateral, and in the latter case 23 specimens out of 29 are midventral. In the Chinese form, CHEN ('31, STEPHENSON) informs STEPHENSON that among 50 specimens, 30 belong to the former case and the remainder to the latter case. So, the ratio of them in both forms, though the number of specimens in each observation are insufficient, is nearly equal.

In the first and last columns, it may be reasonable that they diverge in position of the occurrence. But it is noticeable that in the first column 40 specimens have no genital papillae on both regions preclitellar and postclitellar.

(c) Local variation:

A considerable number of specimens of the Central Korean region have no genital papillae on both regions preclitellar and postclitellar, while, as is clear in Table IX most of the west-northern specimens have both of them. So, only with regards to the genital papillae, the specimens belonging to the latter region are rather closely related to the Chinese form. And, it is also interesting to notice that most of the specimens belonging to the same region have the ventrolateral ones.

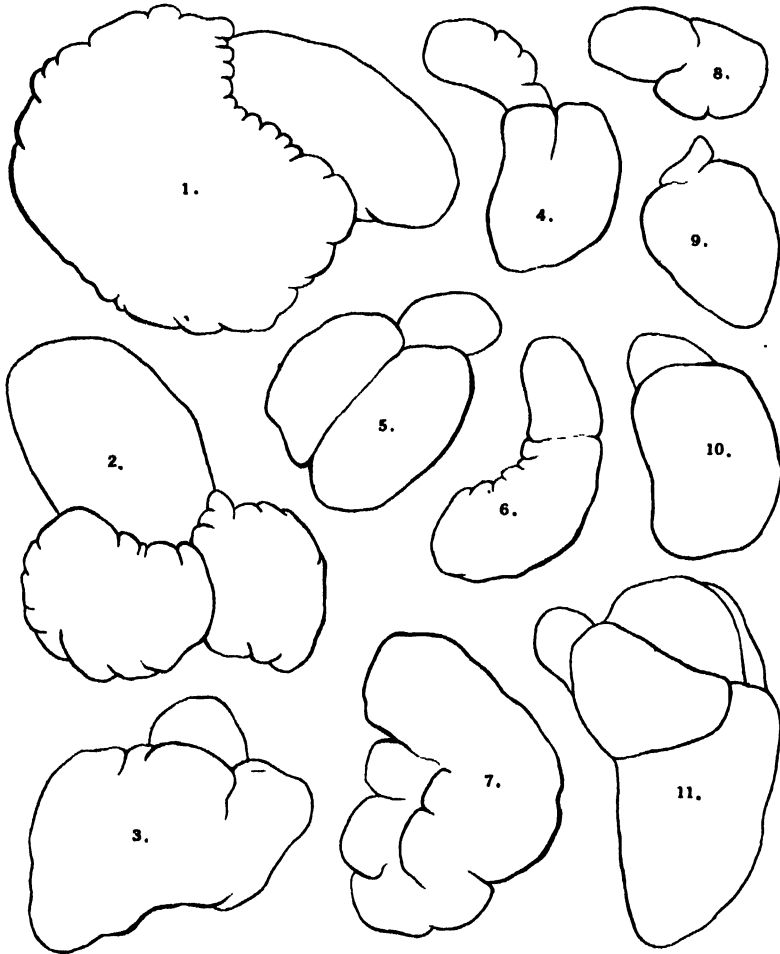
#### INTERNAL CHARACTERISTICS

As to the internal structures I have very little to add to those descriptions given already by previous authors.

The hearts of X are present but are small or sometimes rudimentary, usually empty and bound by the connective tissue to the anterior face of 10/11, as GATES ('35, p. 14) revised the absence of them in CHEN's description ('33).

Seminal vesicles are considerably variable in size, shape and also in texture. Some representative instances are illustrated below. As is seen

in it, its size is so different even in the apparently similarly matured specimens, that only with regards to this organ they appear to be the separated species from each other. In its texture of the surface of the vesicle, one is much tubercular, but the other is smooth. In its shape,



Text-fig. 3. Showing the variation of the seminal vesicles of *Ph. carnosa*. ca.  $\times 9.4$

one is subdivided into two or more, but the other is simple; so, the dorsal lobe is considerably variable, i.e. one is small or indistinct, the other is very much large or moderately large (in size), and ovoidal or rounded or penis-like (in shape), and smooth or rather vesicular (in texture). If a large number of specimens are opened, the variation will increase.

In spite of such considerable variation only one point is noticeable, that the specimens collected from the same locality all have vesicles similar in size, shape and also in texture. For instance, those collected from En-an (also Heijô, Chinnampo, Junsen and Shariin) all have the type of either 1 or 2. Whether or not this noticeable fact is local variation cannot be decided here.

I could not find the spermathecae having the special secondary sacculles occurring on both ampulla and diverticulum, though several cases in which the diverticulum is with the bifurcate ental end were found.

Except for these variations, no further consistent difference could be found between the Chinese and Korean forms; the earthworms used in this study are validly identical with *Pheretima carnosus* (GOTO et HATAI).

Finally, it is a pleasure to record here a debt of gratitude to Professor SHINKISHI HATAI for his kindness in reading this paper. Also, I desire to acknowledge the assistance rendered in the collection of the worms in west-northern region through a grant from the SAITÔ GRATITUDE FOUNDATION (SAITÔ HÔ-ON KAI).

#### POSTSCRIPT

##### *Pheretima* (Ph.) *carnosa* (GOTO et HATAI)

*Locality*: Morioka, Iwate prefecture, northern part of Central Japan.

*Material*: two clitellate specimens (one—posterior part regenerated).

*Description*:

*External characteristics*: Length 220 mm., greatest diameter 7.5 (7.5) mm., number of segments 127. Colouration, dorsally brownish and concentrated anteriorly, ventrally pale, clitellum light brown.

Prostomium epilobous ca.  $\frac{1}{2}$ .

First dorsal pore: (1) That in 12/13 functional but smaller than the next; in 11/12 a slight non-functional dorsal pore-like marking was found. (2) In 12/13 a well-marked but non-functional pore was found; that in 13/14 slightly larger than the former and functional.

Clitellum entire, in XIV-XVI (=3), without setae, dorsal pores, and intersegmental furrows.

Setae beginning on II; ventral ones on III-IX enlarged and widely spaced, in the other segments ventral ones nearly equal to the dorsal in size, the latter may be very slightly more closely set than the former. Setal ring with middorsal break,  $zz=1.2-2.0\ zy$ , midventral break (1) indistinguishable or very slight if present, or (2)  $aa=1.2-2.0\ ab$ , but partly

also indistinguishable. Setal numbers as follows: 32 (33)/III, 51 (55)IX; 14 (14)/SVI, 16 (16)/SVII, 20 (20)/SVIII between spermathecal pores; 18 (17) between male pores; 4 (4)/G<sub>1</sub>, 4 (4)/G<sub>2</sub>, 6 (mr)/G<sub>3</sub>, (18 (17))/G<sub>4</sub>, mr (ml)/G<sub>5</sub>).

Male pores situated ventrolaterally on XVIII, slightly larger than 1/3 of circumference apart, each pore minute, in the centre of a round or oval papilla which is slightly raised. Genital papillae, distinctly raised, circular and centrally slightly depressed, larger than the papillae bearing the male pores. Their occurrence and position are as indicated in the setal relationship. Ventral surface of XVIII is slightly concaved and crescentically formed on both sides.

Female pore single, midventrally on XIV.

Spermathecal pores, 4 pairs in 5/6-8/9, slightly larger than 1/3 of circumference apart, each with an anterior spermathecal papilla. Genital papillae similar, found in male pore region, situated on VIII and IX midventrally.

#### *Internal anatomy:*

Septum 4/5 slightly thickened, 5/6-7/8 very much thickened, 8/9 membranous and shoved back to posterior side of gizzard, 9/10 absent, 10/11-12/13 much thickened, the succeeding septa gradually thinner.

Nephridial tufts thick, attached to the anterior faces of 5/6 and 6/7.

Gizzard lying in 7/8-10/11, fairly large, globular in shape. Intestine beginning to swell in XV (or in the middle part of XV). Intestinal coeca, simple, conical, originating in XXVII, extending as far anteriorly as XXIV or XXIII, both ventral and dorsal margins nearly smooth, or with slight septal constrictions, or ventral margin only with a few light coloured indistinct outgrowths.

Last hearts in XIII. Lymph glands whitish, fairly large, paired in each segment, found on both sides of dorsal vessel behind 15/16 caudalwards.

Seminal vesicles, 2 pairs in XI and XII, small, not meeting middorsally, posterior pair slightly larger than the anterior, each vesicular on surface, with a large ovoidal smooth dorsal primary ampulla (in one specimen, each of the anterior pair subdivided into 3 divisions). Testis sacs in X and XI, moderately large; anterior pair rounded, projecting into X, connected medially with its opposite by a rather broad bridge, posterior pair almost completely fused into a transverse sac. Both pairs in contact with each other. Testis large, round, situated at the anterior inner wall of each sac; funnel also large, situated in usual position. Sperm-ducts on



each side united in XII. Pseudovesicles, 2 pairs, anterior pair relatively large or small, ovoidal, posterior pair much smaller or rudimentary, club-shaped, attached to the posterior faces of 12/13 and 13/14 respectively.

Prostate glands well-developed, each divided into 3 or 4 main lobes, extending from  $XVI\frac{1}{2}$ –XIX ( $=3\frac{1}{2}$ ); duct rather thin but stout, looped in U- or O-shape, distally slightly thicker.

Spermathecae, 4 pairs, last two pairs placed between 7/8 and 8/9. Ampulla heart-shaped or elongated pear-shaped or spatulated, nearly smooth or slightly wrinkled on surface, sometimes slightly zigzagged marginally; its duct stout and long but slightly shorter than the ampulla in length, usually distinctly marked off from the latter. Diverticulum slightly longer or nearly equal to  $\frac{1}{2}$  of the main portion in length, its distal part slender and entally formed a small elongated or rounded swelling.

Accessory glands found corresponding to the external genital papillae, each one roundish and granular on surface.

It is a pleasure to acknowledge my hearty thanks to Dr. SHINRYÔ OHFUCHI, Zoologist, SAITÔ Gratitude Foundation, Sendai, for his kindness in sending the materials essential for the present study.

# EARTHWORMS FROM KÔRYÔ, KOREA

By

SHINJIRO KOBAYASHI

(*Keijô-Daini-Kôtô-Futsû-Gakkô*)

(With fifteen figures)

(Received April 8, 1936)

At the request of Mr. Kiûjirô Susaki, an assistant expert of the Forest Experiment Station, General Government of Chôsen, I identified a number of earthworms which were collected by him for the purpose of his ecological study, from Kôryô (N. L. 37° 45'), Keiki-dô, about 30 K. M. distant from Keijô, Korea. The collection ground is a woodland about 300 M. in mean altitude and collections were made four times, once in each respective month, April, July, September and November, 1934. The entire collections were found to be represented by the 14 forms indicated in the following list ;

- (1) *Drawida nemora*, n. sp.
- (2) *Pheretima* (Ph.) *hilgendorfi* (MICHAELSEN)
- (3) *Pheretima* (Ph.) *koryoensis*, n. sp.
- (4) *Pheretima* (Ph.) *fibula*, n. sp.  
var. *typica*, n. var.
- (5) var. *ranunculus*, n. var.
- (6) *Pheretima* (Ph.) *serrata*, n. sp.
- (7) *Pheretima* (Ph.) *monstrifera*, n. sp.
- (8) *Pheretima* (Ph.) *susakii*, n. sp.  
var. *typica*, n. var.
- (9) var. *patina*, n. var.
- (10) *Pheretima* (Ph.) *vallis*, n. sp.
- (11) *Pheretima* (Ph.) *bitheca*, n. sp.
- (12) *Allolobophora japonica* MICHAELSEN ?
- (13) *Eisenia rosea* (SAVIGNY)
- (14) *Bimastus* sp.

As is clear in the list, the genus *Pheretima* is most predominant in this region, but in the individual number *Drawida nemora* is most abundant, followed by *Pheretima hilgendorfi*, and Lumbricid worms are small in number. Of these 14 forms, except *Pheretima hilgendorfi* and last

three Lumbricid worms, all seem to be new to science; and of these ten new species, only one species *Pheretima monstrifera* has hitherto been also collected from the other localities a good distance apart.

By his collections the approximate seasonal development in each species at this region was made clear to some extent, but on this account, since the most of the specimens collected in September and November were very poorly preserved, and more, unfortunately some species of April and July collections had been mixed together, future studies are necessary. However, at this place, it may be said that each of the genus *Pheretima* attains the fully grown condition after August in this region.

A very interesting worm *Pheretima bitheca* has transversely two grouped pairs of the spermathecae. I placed it into the sub-genus *Pheretima*, according to MICHAELSEN's systematics in having the paired moderately well-developed intestinal coeca, as in *Pheretima bleckwenni*.

In the sub-genus *Pheretima*, with the exceptions of *Pheretima hilgendorfi* and *Ph. monstrifera* which have hitherto been also collected from the other localities mentioned above, the structure of the testis sacs of all species are characteristic, annular sacs around the oesophagus, so I made a slight microscopical anatomy on such organs of *Pheretima koryoensis*, as a representative of the other.

*Drawida nemora* has also not yet hitherto been collected from any other localities.

For preservation Mr. SUSAKI used a mixture of alcohol 6, chloroform 3 and acetic acid 3. These earthworms are preserved in the Forest Experiment Station.

Except for the schematic drawings, all figures are camera lucida drawings.

Acknowledgements are due to Dr. TOKUJI KABURAGI, the director of the Forest Experiment Station, General Government of Chôsen (Korea) and Mr. KIÚJIRÔ SUSAKI for placing their material at my disposal; to Dr. SHINKISHI HATAI for his kind direction; to Dr. HARUJIRÔ KOBAYASHI and to Dr. TAMEZÔ MORI for their kind direction and encouragement given me throughout this work; and to Dr. KAHORU NAKADA for his valuable suggestions on the sectioning of the worm.

Family **MONILIGASTRIDAE**Genus **DRAWIDA** MICHAELSEN***Drawida nemora*, n. sp.**

A number of specimens, collected in April and July; most of them apparently mature.

*External characteristics.*

Length 65–120 mm., greatest diameter (around clitellar region) up to 5 mm., number of segments 165–200; dimensions of several specimens picked up at random as follows: 87 × 4.5 mm. and 186 (segments), 92 × 4.5 mm. and 198, 87 × 5 mm. and 192, 82 × 4.5 mm. and 188, 70 × 4 mm. and 168, 100 × 5 mm. and 189, 110 × 4.5 mm. and 198. Anterior-most portion of the body pointed and I–clitellum gradually becoming larger in diameter, making the region rather conical (Fig. 1. 1). Prostomium probolous, narrow but long, separated from first segment by a groove and projecting into buccal cavity and extending inward to slightly over I/II. Length of segment taken from a specimen picked up at random as follows: IV–1.5 mm., VII–2 mm., IX–1.8 mm., XIII–1.3 mm., XX–0.5 mm., C–0.3 mm., last seventh segment–0.2 mm. Permanent secondary annulations may be absent. Colour, dorsally dark bluish, ventrally yellowish grey, clitellum similar to ventral surface.

Dorsal pores absent.

Clitellum in X–XIII (=4), distinct in most specimens collected in April and July, glandular part appearing thicker and slightly swollen, distinctly distinguished from its neighbouring segments by its glandular skin and different colouration, sometimes the glandularity and different colouration dorsally, slightly extends over on IX and XIV anteriorly and posteriorly, ventrally its surface is slightly less glandulated.

Setae beginning on II; small and closely paired; setal lines slightly elevated from the general surface, especially distinct on the outer bundles on the region posterior to clitellum; antecitellar, sometimes also clitellar ones, very slightly smaller than the rest; *aa* usually slightly wider than *bc*, *ab* nearly equal to or slightly wider than *cd*, *dd* nearly equal to  $\frac{4}{7}$ – $\frac{5}{8}$  of circumference, on spermathecal region  $aa=1\frac{1}{4}$ – $1\frac{1}{7}$  *bc*,  $ab=cd$ ,  $dd=\frac{5}{8}$  of circumference, on clitellar region  $aa=1\frac{8}{25}$ – $1\frac{1}{3}$  *bc*,  $ab=cd$ ,  $dd=\frac{13}{21}$  of circumference, on middle portion of the body  $aa=1\frac{2}{5}$ – $1\frac{5}{12}$  *bc*,  $ab=cd$ ,  $dd=\frac{4}{7}$  of circumference; each seta sigmoid, curved at both ends in a weak S-shape.

Nephridiopores, in most cases scarcely visible under high magnification, in line with *d*.

Male pores (Figs. 1. 1 and 2), one pair of very large transverse slits in X/XI, between *b* and *c*, about 8/21 nearer to *b*, representing the secondary male pore in which is situated a conical penis usually not visible externally; each slit largely opened on top of light-coloured, not sharply conical protuberant tubercle of a epidermal thickening of both posterior and anterior about 1/3 (incomplete annulations) of X and XI, its base transversely oval but not distinctly demarcated and in most cases the intersegmental furrow faintly runs on each side.

Female pores, one pair of transverse slits, minute but rather easily visible in well-preserved specimens under the lens, in XI/XII, strictly saying on anterior border of XII in line with *b*.

Spermathecal pores, one pair of minute, longitudinal slits, in VII/VIII, close to the posterior border of VII just medial to *c* or line with *c*, in most specimens where the region is slightly depressed in a crescent shape, sometimes anterior margin of the crescent slightly swollen and formed an anterior lip facing the pore (Figs. 1. 1 and 3).

Genital papillae present on VI-XIII, often externally invisible, sometimes totally absent; their arrangement seems to be regular, having one to three pairs near the spermathecal pores and one or two pairs on every segment of or some of VI-XIII, either one pair just medial to *a* or to *c*, or two pairs combining in both of the two cases; in a large number of specimens the additional ones are found, mostly not symmetrically, just lateral to *d* or ventromedially, but never occur on dorsal surface; each papilla usually rather indistinct, small, whitish or pale, circular tubercle which is very slightly protuberant from the general surface, sometimes it is slightly sunken into the body wall, more frequently externally invisible, but, after dissection, oval glands are found internally where the papillae should be present externally, — (somewhat resemble those of *Dr. hattamimizu* HATAI), (Fig. 1. 1); seldom, totally absent externally and also internally.

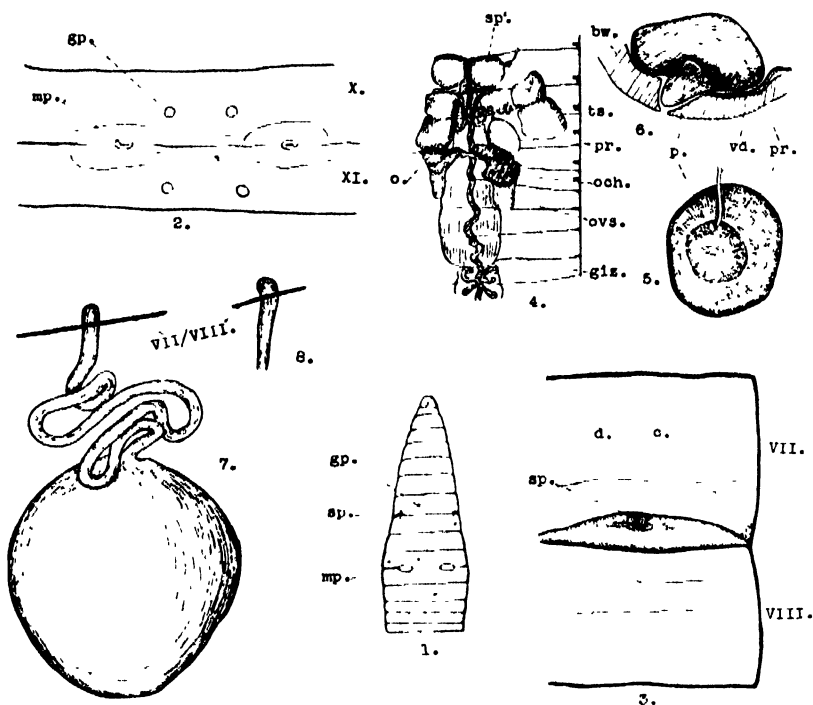
#### *Internal anatomy:*

Septa V/VI-VIII/IX thickened, of these posterior two much thickened and the last one in middle part of IX, the remaining septa all thin and membranous, IX/X and X/XI in posterior part of X and XI respectively, XI/XII fused with X/XI dorsally forming ovarian chamber, posterior septa to this normal in attachment.

Pharyngeal glands, moderately thick and dividing into several transverse layers in III and IV, connected with body wall by ligaments.

Nephridia, meganephridial, absent in first two and last few segments and also in XII, usually present in the rest; those in VI-IX large, in X and XI rather small; nephridial vesicle long, extending to dorsal side or even over-lapping with that from opposite side, about 3-6 mm. in length, its tubule convoluted lying upon posterior face of septum with only its minute nephrostome protruding through septum into preceding segment.

Gizzard brownish or golden in colour, generally smooth and shining on surface, the middle ones thick ring-shaped, and first and last ones



Text-fig. 1. *Drawida nemora*, n. sp. 1. Ventral view of the anterior part of the body, (free hand drawing). 2. Ventral view of X and XI. ca.  $\times 12.6$ . 3. Ventrolateral view of VII and VIII, with spermathecal pore, ca.  $\times 24$ . 4. Dissection of the internal organs, with dorsal body wall removed (not showing hearts, nephridia and glands of papillae) (free hand drawing). 5. Ventral view of prostate and penis with vas deferens (separated off from the body), ca.  $\times 12.6$ . 6. Lateral view of 5, with body wall, (cleared), ca.  $\times 12.6$ . 7 and 8. Spermatheca and ectalmost portion of the spermathecal duct, ca.  $\times 22.2$ . bw. body wall, giz. gizzard, gp. genital papilla, mp. male pore, o. ovary, och. ovarian chamber, ovs. ovisac, p. penis, pr. prostate, sp. spermathecal pore, ts. testis sac, vd. vas deferens. VII/VIII. position of intersegmental furrow VII/VIII (Sp. Spermatheca).

somewhat cup-shaped and sometimes one of these two or both of them less muscled than the middle ones, all closely coalesced one with another, in the majority of specimens four gizzards in XII–XV, sometimes five in XII–XVI and three in XIII–XV (Fig. 1. 4).

Dorsal vessel rather large in calibre; blood sinuses found behind last gizzard; lateral hearts, 4 pairs in VI–IX connecting dorsal and ventral vessels; commissural vessels large in calibre, paired in IX and X connecting with latero-oesophageal vessels, all free from septa and lying upon oesophagus.

Testis sacs, one pair, each suspended with its slightly smaller part in IX and larger part in X (sometimes, each part in IX and X nearly equal); about middle part neck-likely constricted, especially marked on the side facing oesophagus, perhaps due to attachment of septum, nearly rectangular in shape as a whole, about 4–6 mm. in anteroposterior length, 2–2.8 mm. in width, anteroposterior length of part in IX about 1.8–2.2 mm., part in X 2–4 mm., yellowish white and granular on surface. Testis, large, nearly circular, seminal funnel whitish and shiny just behind the testis on ventral side of each sac. Sperm-duct long, moderately closely coiled, running in front of IX/X around last heart, piercing the septum into X and touching nephridium of X to a certain degree and finally passing under the anterior side of prostate to connect directly with the anterior and basal portion of penis. Prostate thick oval or circular disc, sessile on parietes, occupying, mostly, about  $1\frac{2}{3}$  segments in X and XI remaining for a trifle length anteriorly and posteriorly, sometimes the dorsal face of the disc is slightly rounded like a cake-urchin, about 1.2–2.5 mm. in diameter, yellowish white and usually smooth on surface; a small penis not sharply conical is deeply buried with its rather wide base into the ventral centre of the prostate, is enclosed within and almost fully occupying the penial pouch (external male tubercle); on top of the penis a large transverse slit-like primary pore opens (Figs. 1. 4–6).

Ovarian chamber in XI, formed by septa X/XI and XI/XII, both septa entirely fused dorsally but rather widely separated ventrally, also closed around oesophagus in an inverted U-shape. Ovaries, one pair, large and rosette in appearance, situated at anteromedian corner of chamber. Ovisacs, one pair, extending slightly backwards from dorsal side of XI/XII, lying on both sides of gizzards, in XII–XIII or XIV, voluminous, flattened and inverted bell-shaped, moderately deep yellow, filled with ripen ova, small and posteriorly much narrower compared to its rather large mouth if whitish and little sex products contained,—(unfortunately, as the collector

had mixed the specimens of both April and July collections, it is not clear whether the specimens having voluminous sacs belong to the April or July collection). Anterior face of XI/XII thickened with glandular ridges, two thick ridges extending from mouth of sac down to median side of each ovarian chamber, median one thicker and stouter, whitish, turned outwards to body wall to meet lower part of lateral ridge, the groove thus formed by both ridges communicating ovisac and external opening serving as an oviduct.

Spermathecae, one pair; ampulla and duct wholly lying upon posterior face of VII/VIII; ampulla thin-walled, large, round, of about 1-2 mm. in diameter, internally filled with whitish coagulated sex products in all cases examined (preserved specimens); from its lower side rises a thin and long (5-8.5 mm.) duct moderately sharply marked off, running with loose and irregular twists and finally becoming nearly straight to enter directly into the parietes without artial dilation, its ectalmost portion burried in the parietes usually slightly extending beyond intersegmental furrow VII/VIII into VII and is a trifle thicker than the rest but often without enlargement (Figs. 1. 7 and 8).

*Remarks :*

No spermathecal atrium and circular disc-like prostate relate the present species perhaps to *Dr. rara* GATES, but by the same author's reports (spermathecal atrium traceably present (1926 and 1931) or its presence is not clear (1933)), their similarity is much decreased; and more, it differs from the latter in genital markings, position of the ectalmost of the spermathecal duct, position of gizzards, and body size. Also, it is easily distinguished from the other Korean *Drawida* forms, by (1) external general form of the body, (2) large, transverse slit-like secondary male pore, situated on top of the tubercle which is not sharply conical and is formed by thickening of both posterior and anterior borders of X and XI, (3) disc-like prostate with smooth surface; vas deferens passing under the anterior side of prostate to connect with the anterior and basal portion of small penis which is deeply burried with its rather wide base into the ventral centre of the prostate, (4) spermathecal pore situated on posterior border of VII (which is, in most cases, slightly depressed in a crescent shape), minute longitudinal slit in line with or just medial to c; no spermathecal atrium, and ectalmost portion of the spermathecal duct which is burried in the parietes, terminates anteriorly often a trifle passing over the intersegmental furrow VII/VIII, (5) mostly occur, small, whitish or pale genital papillae, in most cases one pair, sometimes with additional ones, on



every segment of or some of VI–XIII normally just medial to either *a* or *c* and mostly concentrated in one to three pairs near the spermathecal pore.

The present species is most predominant in this region in number.

Family MEGASCOLECIDAE  
Subfamily MEGASCOLECINAE  
Genus PHERETIMA KINBERG  
Subgenus PHERETIMA MICHAELSEN

*Pheretima* (Ph.) *hilgendorfi* (MICHAELSEN)

The present species is, in the sub-genus, most predominant in this region. And, I reported already (1934) that it is distributed here. A detailed description of the species will be made in the near future.

*Pheretima* (Ph.) *koryoensis*, n. sp.

About 20 acitellate and 25 clitellate specimens (most of the specimens collected in September and November are poorly preserved).

*Description of the type specimen* (well-preserved September specimen).

*External characteristics:*

Length 160 mm., greatest diameter 12 mm., number of segments 128. Colour, dorsally dark brown, clitellum dark grey.

Prostomium epilobous about  $\frac{1}{2}$ .

First dorsal pore in XII/XIII.

Setae beginning on II and rather small; setal ring with slight mid-dorsal break on segments of middle portion of the body, and without midventral break; anteclitellar setae smaller, especially the ventral and lateral setae of V–XI, than the postoclitellar, generally the ventral setae are smaller and more closely set than the dorsal; approximate setal numbers are as follows: 68/VI, 76/VII, 72/VIII, 78/XIII, 82/XX, 38 (VI), 42 (VII), 40 (VIII) between spermathecal pores, 11 between male pores.

Clitellum entire, extending from XIII/XIV–XVI/XVII, without setae, dorsal pores and intersegmental furrows.

Spermathecal pores, 3 pairs, minute and simple, opening barely recognizable, anteriorly located on VI, VII and VIII, quite close to the intersegmental furrows, about  $\frac{4}{7}$  of the circumference apart.

Female pore single, on XIV midventrally.

Male pores ventrolaterally on the setal line of XVIII; each situated on a large, markedly protuberant, light-coloured, transversely placed oval disc; each disc extending from XVII/XVIII to XVIII/XIX, but these two intersegmental furrows are actually slightly displaced anteriorly and posteriorly by pushing of the disc, transversal length of disc longer than the distance between two discs, the former about 4.7 mm., the latter about 2.5 mm., the anteroposterior length about 3.0 mm., on the surface of the disc present a distinct groove which may be said to be of a V-shape fallen to the lateral side; discs probably slightly retractile into the parietes. (The male pores are externally not visible. The approximate location of the pore can, however, be determined by carefully pulling the prostatic duct from the parietes after removal of the longitudinal musculature. This procedure exposes an aperture slightly median to the centre of the disc on the edge of a fallen V-shaped groove). On XIX, the outer layer of both sides behind the discs are thickened, light-coloured, setae on these thickenings becoming inconspicuous and scarcely visible. The region between the discs is much wrinkled longitudinally, where 11 setae are planted (Figs. 2. 1).

There are no other genital markings elsewhere.

*Internal anatomy:*

Septum IV/V thickened, V/VI-VII/VIII much thickened, VIII/IX and IX/X absent, X/XI and XI/XII much thickened, the succeeding septa gradually becoming thinner.

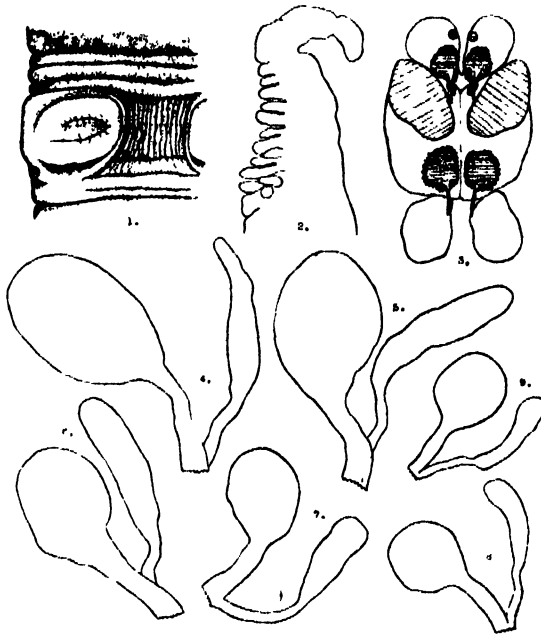
Pharyngeal glands not bulky, as usual.

Nephridia, moderately thick tufts in V and VI, both integumental and septal not conspicuous.

Gizzard moderate sized, bell or barrel-shaped, occupying about from VII/VIII-X/XI, nearly smooth and shining on surface. Intestine beginning to swell in XV. Intestinal coeca originate in XXVII, extending as far anteriorly as XXIII, where they are bent under the intestine but long enough to reach into XX; each coecum on the ventral margin with about a dozen serriformed outgrowths (Fig. 2. 2).

Commissural vessels in IX terminated on gizzard dorsally, those in X asymmetrical, one loop larger and complete, the other rudimentary; hearts, 4 pairs in X-XIII, all moderate and nearly equal in calibre, first two pairs enclosed in the corresponding testis sacs; dorsal vessel rather small in calibre, but moderately enlarged in the region of X-XIV; lymph glands whitish, spatulated in shape, distinct, found on both sides of dorsal vessel, behind XIV/XV backwards, especially much distinct behind coecal segment.

Testis sacs, annular, two in X and XI. Anterior pair of sacs, large, compact, fused both dorsally and ventrally forming a ring with antero-lateral rounded horns, which dorsally appears to be U-shape and ventrally V-shape; posterior pair also form an ample, ring-like sac, dorsally and ventrally fused; both ring-like sacs enclose hearts, dorsal and ventral vessels, and gut, posterior sac more enclose the seminal vesicles of the segment. These two sacs separated from each other by the septum X/XI. Seminal vesicles, two pairs, small, in XI and XII; each vesicle of the anterior pair



Text-fig. 2. *Pheretima koryoensis*, n. sp. 1. Ventral view of XVIII, ca.  $\times 4.2$ . 2. Intestinal coecum, ca.  $\times 7.3$ . 3. Ventral view of the anterior male organs (free hand). 4-6. Spermathecae of type specimen, 4-VIII, 5-VII, 6-VI, ca.  $\times 7.3$ . 7-8. Spermathecae of July-clitellate specimen, but with either setal pits or vestigial setae on clitellum, ca.  $\times 7.3$ .

which is enclosed within the testis sac, nearly oval, with a small smooth dorsal primary ampulla, each of the posterior pair situated posteriorly to the large testis sac of XI as a small hanging body, oval, slightly smaller than that of the anterior pair, with a small smooth dorsal primary ampulla. Testis and funnel enclosed in the testis sacs; testis of X disc-like, attached to the anterior inner wall of the sac near to the midventral line, funnel

situated immediately behind the former; testis of XI somewhat oval, attached to the anterior inner wall of the large sac rather near to midventral line, funnel situated behind testis, slightly more apart than in the former case; two funnel ducts on a side united at nearly end of XIII. Pseudo-vesicles, one pair, attached to the posterior face of XII/XIII, large, whitish and ovoidal (Fig. 2. 3).

Prostate moderate sized, occupying about XVII-XIX; gland indistinctly divided into about 4 main lobes, these into much smaller lobules, supported by much fine connective fibres; duct stout, moderately thick, nearly straight, ectally becoming gradually thicker.

Spermathecae, 3 pairs in VI, VII, and VIII; each ampulla dorso-ventrally flattened oval; spermathecal duct slightly shorter than the ampulla in length, not sharply marked off from the latter; diverticulum, that in VIII shorter but both of VI and VII longer than the combined length of the duct and ampulla, the ectal one third tubular and slightly winding, ental remaining forming a large widening (Figs. 2. 4-6).

#### Remarks:

April specimens all are aclitellate; July specimens mostly clitellate, but the majority do not seem to be fully grown, for the following points: (1) each clitellar segment with either vestigial setae or setal pits, especially distinct ventrally on XVI; clitellum with slight intersegmental furrows and the positions of the dorsal pores indicate the non-functional pore-like markings. (2) spermathecae are much smaller than those of the September and November specimens (Figs. 2. 7-9).

Length varies from 110-167 mm., greatest diameter 7-12 mm., number of segments from 105-128. The middorsal break in the setal line is found on the segments of about XXX-LXX, with varied width. The approximate setal numbers of a few specimens are given in the below table.

II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XVII	XX	XXX	MP	SP (VI)	(VII)	(VIII)
41	50	54	58	64	67	71	70	68	72	76	70	78	75	75	10	38	42	44
33	48	55	55	62	65	74	71	77	74	72	83	82	86	86	12	36	39	42
39	53	61	64	68	76	72	76	74	78	76	78	78	82	80	10	38	42	40
46	52	63	62	68	75	74	75	76	80	80	76	80	79	76	10	38	40	42

MP.....between male pores.

SP (VI), etc.....between spermathecal pores.

The spermathecal pore is a distinct characteristics for the present

species; it is located more than one half of the circumference apart, and minute and barely recognizable.

Externally, the male pore discs on XVIII are the most distinct characteristics; even in the acitellate specimens, the positions of the discs are largely elevated and light-coloured. In the clitellate specimens, the transversal length of the disc varies from about 2.5–5 mm., and is always longer than the distance between two discs; 7–12 setae are intervening between them.

The prostate is moderate or small in size; duct usually nearly straight, but sometimes slightly bent anteriorly.

In most species of the genus *Pheretima*, the testis sacs are ventral with small chambers in segments X and XI, as described by BERGH (1886). But, occasionally as in *Ph. elongata* and *Ph. bicincta*, they are much ample in extent, and contain the hearts, dorsal vessel, and oesophagus, together with the seminal vesicles, if any, of that segment (STEPHENSON, 1930). GATES lately (1932) reported that in *Ph. elongata*, both testis sacs in X and XI are not always dorsally fused, but in half of all specimens those of X and XI come into contact and end against the dorsal blood vessel. In several Chinese forms reported separately by MICHAELSEN (1910), GATES (1935) and Y. CHEN (1933), the testis sacs of XI include the corresponding seminal vesicles, but they all, except *Ph. abdita* GATES (1935), are not annular testis sacs, but U-shaped or horse-shoe-shaped ones, either ventrally or dorsally not completely fused. GATES described (1932) "Perhaps more than often has been realized the vesicles of XI are included within the testis sacs of that segment. This is true at least of *Pheretima posthuma*, an old and familiar species in which this characteristics has not hitherto been noted." In fact, such forms are gradually increasing in number by the intensive works of the zoologists in many localities such as India, Burma, China, etc.

During the present work, I encountered the fact that all species of the sub-genus *Pheretima*, except two, *Ph. hilgendorfi* and *Ph. monstifer*, have the characteristic annular testis sacs dorsally and are also ventrally completely fused. I made a slight microscopical anatomy of *Ph. koryoensis*, as a representative of the species having such organs. The following is the description.

1. Septa of the anterior male genital region.

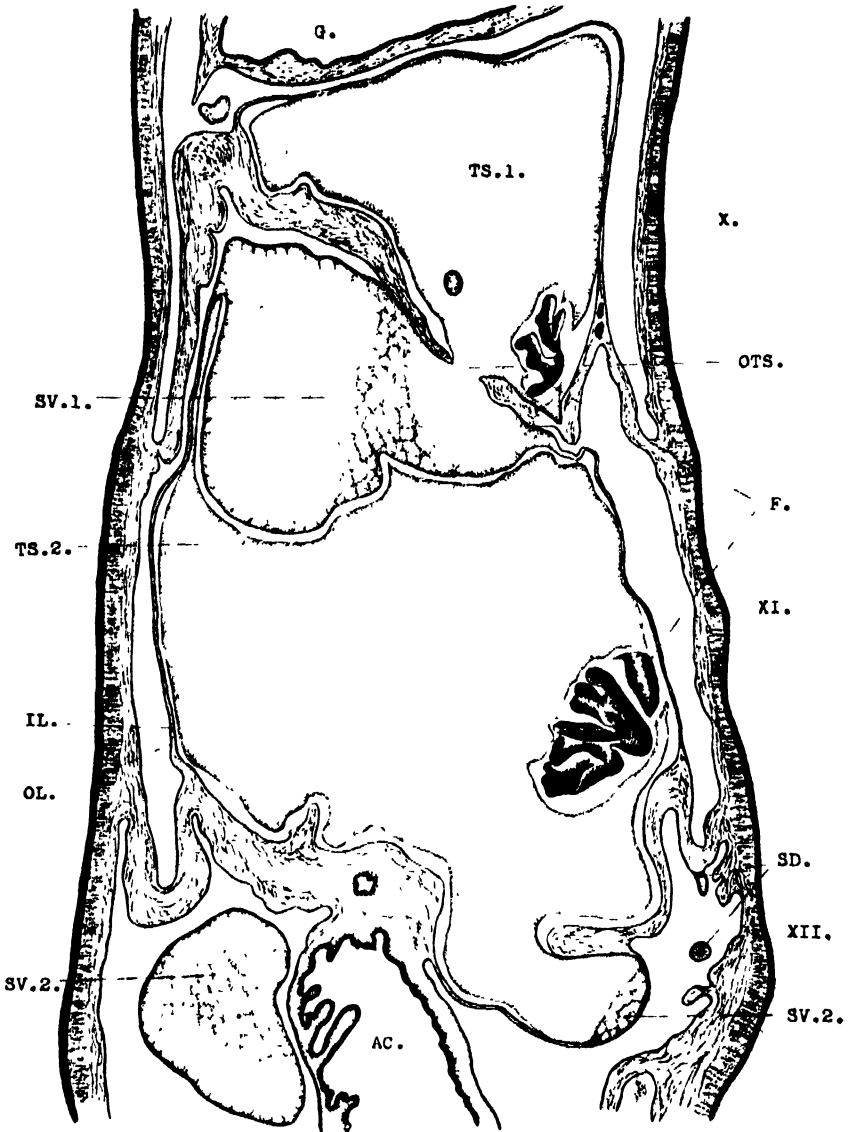
A gizzard extends from the septum VII/VIII to about X, and septum VIII/IX is rudimentary and projects ventrally from the parietal wall as some faint muscular fibres, but actually it may be said to be absent,

IX/X absent, X/XI and XI/XII are much thickened, muscular and somewhat funnel-shaped, and both of them are dorsally still thicker. The septum X/XI does not attach correspondently to the intersegmental furrow, i. e. dorsally it extends anteriorly quite closely to the parietal wall, as far anteriorly as near the posterior margin of the gizzard (about one segment anteriorly displaced), and then as it reverses, it extends to the posterior and inner direction obliquely towards the gut, and reaches the ventral parietal wall, where it is almost correspondent to the intersegmental furrow, and directs again anteriorly for a short distance, until it connects with the ventral parietal wall; so the inner cavity of XI is very large and actually occupies about one and a half segments; in vertical section the septum appears somewhat N-shape. Dissecting and separating the anterior male organs from the body, that septum appears to be dorsally a compressed U-shape and ventrally a V-shape. The septum XI/XII is attached almost in normal position, though the connecting portion with the peripheral parietal wall extends posteriorly for a short distance, forming a funnel there.

The septum consists of two layers of flattened peritoneal cells, which enclose the muscular fibres, connective tissue and blood vessels.

## 2. Testis sacs.

In general, the covering layers of the testis sacs and seminal vesicles resemble those in *Lumbricus terrestris* described by R. HESSE (1894). In the vertical section, the layer of the flattened peritoneal cells lining the anterior face of septum X/XI is prolonged nearly as far anteriorly as the posterior margin of the gizzard. The origin of the prolongation of the layer occurs in two parts, one near the peripheral parietal wall and the other near the outer surface of the gut. By a such manner, as an annular ample anterior testis sac is formed, its anterior margin is free or independent upon any organ and is bluntly rounded. The similar layer of the posterior face of the same septum and that of the anterior face of septum XI/XII forms with their continuance an annular ample posterior sac, so it extends from septum to septum occupying the whole of that segment. The wall of the sac, in which both the portion parallel to the peripheral parietal wall and anterior free part of the anterior sac consist of doubled flattened epithelia, and between them is present a thin layer of connective tissue. The connective tissue consists of muscular fibres, sometimes with blood vessels. In doubled flattened epithelia, the outer layer is naturally derived from near the portion of the septum connecting with the peripheral parietal wall ("*parietal origin*"), separating itself from



Text-fig. 3. *Pheretima koryoensis*, n. sp. Vertical section; showing the anterior male organs, ca.  $\times 18$ . AC., alimentary canal; F., funnel; G., gizzard; IL., inner layer of the wall of the testis sac, "septal origin"; OL., outer layer of the wall of the testis sac, "parietal origin"; OTS., opening of testis sac to seminal vesicle; SD., sperm-duct; SV. 1 and 2., seminal vesicle; TS. 1 and 2., testis sac (with sexual products).

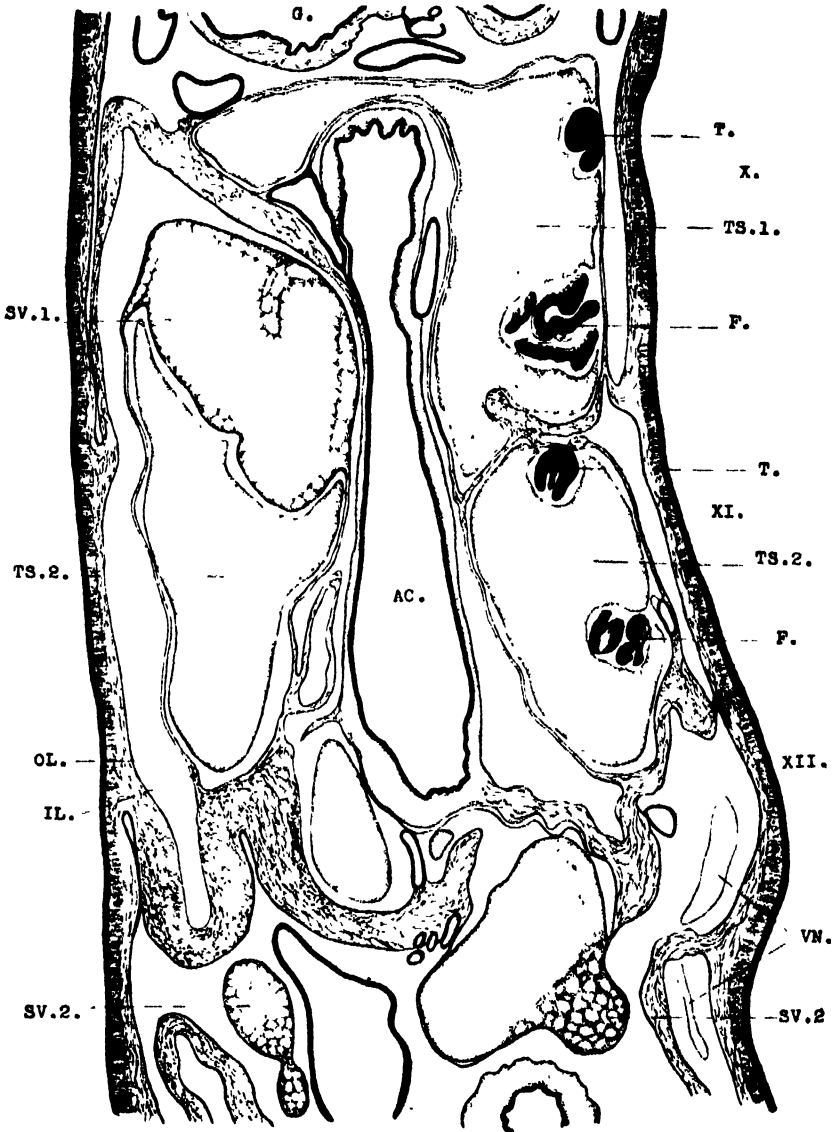
the ground tissue of the septum, the inner layer is derived from the layer lining the middle portion of the septum or the surface of the septum itself ("septal origin"), and the former is united with the latter to form a sac; in vertical section their relation is distinctly recognizable. The wall of the sac, in which the portion surrounding the alimentary canal consists of similar doubled layers; it is very closely lined or surrounds the outer wall of the gut, but its middorsal and midventral portions are not directly or closely lined, since the layer in those two portions is lined or encloses the dorsal and ventral, and lateral- and supra-oesophageal blood vessels together with the gut.

Apparently both testis sacs are opaque white (in preserved specimens) and massive like vesicles, and filled with much sexual products. Anterior sac, when seen from the ventral side, appears to be a massive-V-shape, and its two limbs are rather broad and bluntly rounded, and its posterior midventral portion is comparatively so broad, though it is of less width than the limbs, that it may be recognized even megascopically, communicating the limbs with each other in this portion; anterolateral portion of each limb buldges dorsally around the sides of the gut to communicate dorsally with their opposite one; dorsally it appears to be a compressed massive U-shape, and its middorsal portion is less broad than the midventral, and its two limbs are slightly less broad than the ventral. Even in the specimens with incomplete clitellar glandularity, it contains sexual products, especially much filled in the anterior rounded ends; in the preserved specimens, they can be pulled out from those rounded ends of the sac in an opaque whitish large masses, which resemble appearance of seminal vesicles; from such fact, I remember here GATES' saying on the seminal vesicles of X in *Ph. posthuma* which has been considered to have the vesicles in that segment, missing the sexual products as vesicles. Within such a testis sac, are included the hearts of that segment, the testes and funnels, and also the gut, dorsal and ventral blood vessels in their courses of that segment. The relationship of the sac to the other organs are distinctly visible in cross section, and in some extent it may be detected even under the dissecting microscope, for the sac is ample in extent.

Posterior testis sac is also an annular ample sac extending from septum to septum and fully filled with sexual products, so it appears to be a single cylindrical opaque whitish mass. Its appearance is different ventrally from dorsally, according to the actual situation of the anterior testis sac or the corresponding septum. Its ventral length is shorter than



the dorsal, and along the whole length of its midventral portion runs an indistinct linear depression immediate along the inner side of the ventral blood vessel. In line with this depression the midventral portions of the

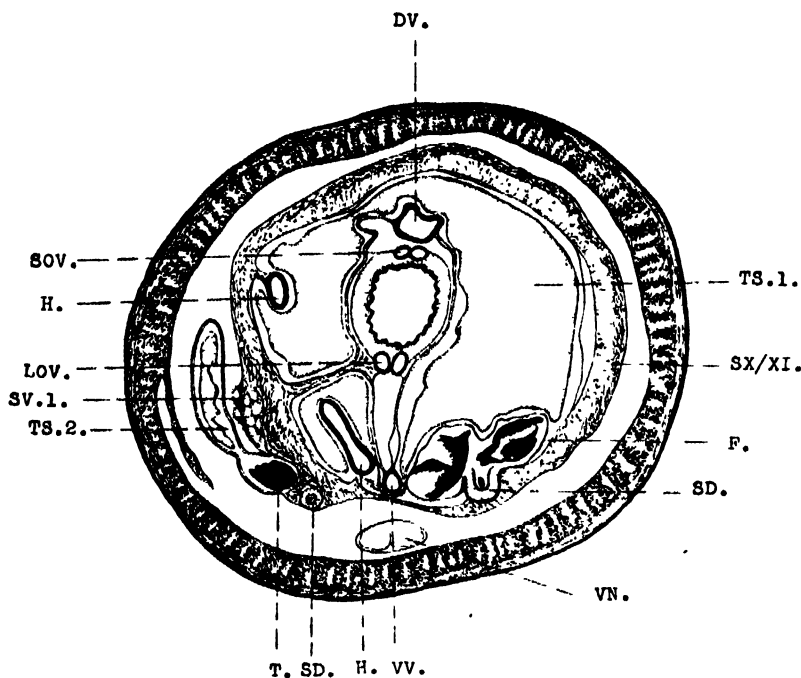


Text-fig. 4. *Pheretima koryoensis*, n. sp. Vertical section; showing the anterior male organs, ca.  $\times 18$ . T., testis; VN., ventral nerve cord; the others as in Text-fig. 3.

septa are also very slightly depressed, sometimes that portion of each septum consists of only two layers of peritoneal cells lining both faces of a septum, having no ground tissue between them. Both midventral depressions of the sac and of septa may have some relation with each other. Within a sac of horse-shoe-shape, are included the seminal vesicles and hearts of that segment, as well as testes and funnels, the gut, and dorsal and ventral blood vessels in their courses of that segment. In most cases, the dorsal surface of the sac, as well as the ventral, is nearly smooth and is filled dorsally with much sexual products. When the sexual products are little or not fully covering the dorsal blood vessel, its surface is slightly uneven, since the wall of the sac is attached to the seminal vesicles and dorsal blood vessel.

### 3. Seminal vesicles.

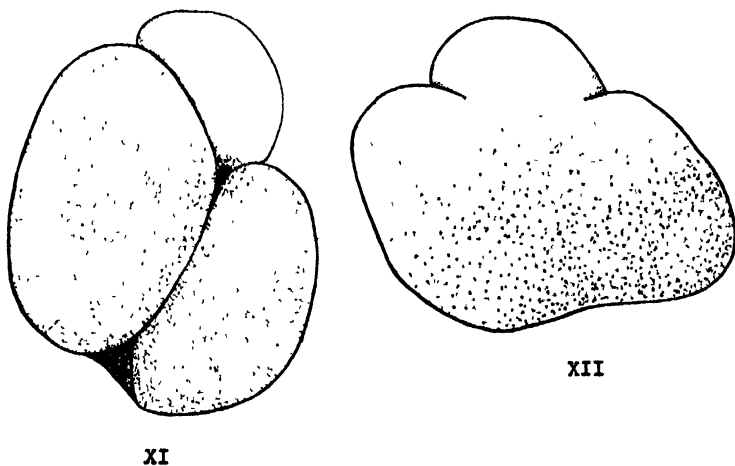
There are two pairs of comparatively small seminal vesicles in segments XI and XII. The anterior pair is attached to both sides of the posterior



Text-fig. 5. *Pheretima koryoensis*, n. sp. Cross section (slightly sagittal), through anterior funnel and posterior testis, ca.  $\times 15$ . DV., dorsal blood vessel; H., heart; LOV., lateral oesophageal blood vessel; SX/XI., septum X/XI; SOV., supra-oesophageal blood vessel; VV., ventral blood vessel; the others as in Text-fig. 3.

face of X/XI, being included within the posterior testis sac, and is nearly equal to or slightly larger than the posterior. The posterior pair is attached to a similar position on the septum XI/XII and is situated near the sides of the gut, sometimes it may cover the dorsal blood vessel, and always slightly pushes XII/XIII posteriorly.

Each vesicle of the anterior pair is a moderate sized dorsal lobe and ellipsoidal in shape. Along its long axis (slightly diagonal and antero-posterior axis) on both dorsal and ventral surfaces runs, a comparatively deep incision with a little connective tissue, so the whole vesicle body is apparently subdivided by it into two ellipsoidal bodies, or these two bodies appear to be cemented by the connective tissue. One of these is slightly larger than the other, and by its long side it is tangentially communicated in the lateroventral portion with the corresponding testis sac. Between



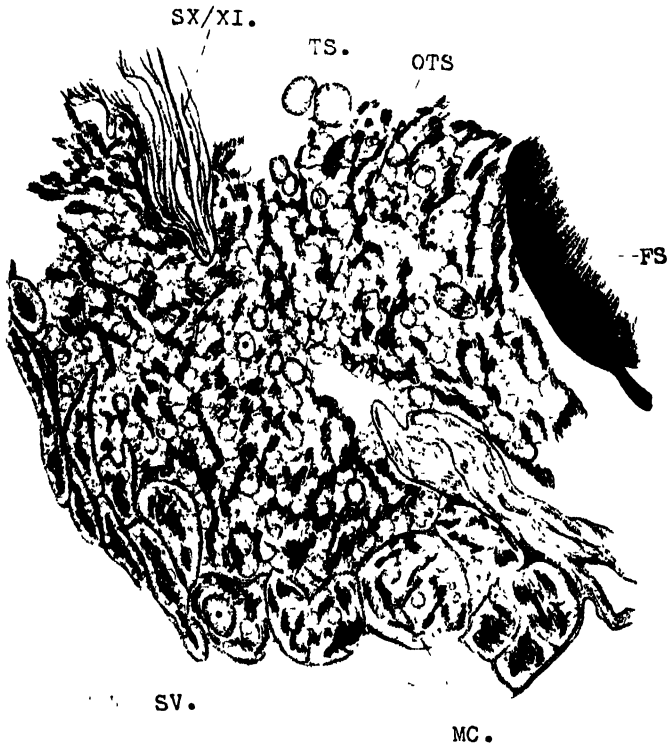
Text-fig. 6. *Pheretima koryoensis*, n. sp. Dorsal view of seminal vesicles, ca.  $\times 10.4$ .

these two bodies, a moderately sized ellipsoidal dorsal primary ampulla is apically present. In most cases, the ampullae are situated rather close to the middorsal line. The vesicles of the anterior pair are variable in size. In most cases, the relationship of its size to the sac is almost as in the figure, but sometimes they are so large as to almost occupy the whole sac.

Each vesicle of the posterior pair is somewhat ellipsoidal but slightly flattened in shape, and with a somewhat ellipsoidal, moderately sized dorsal primary ampulla which is, in most cases, slightly sunk into the body, and is communicated ventrolaterally with the corresponding testis

sac by its anterior, slightly narrowing, flattened end. The posterior pair does not show marked variation in size. The vesicle body of the anterior pair appears to be vesicular, and the ampulla is nearly smooth on the surface. The vesicle body of the posterior pair appears to be also vesicular, and sometimes on its dorsal surface dark brownish pigments are much dotted, but the ampulla is nearly smooth and always lacks such dotted pigments on the surface.

The vesicle is covered by a thin layer of the peritoneal cells of the septum, as well as the testis sac. But, the great part of the latter is naturally of the doubled layers as already described, while the wall of



Text-fig. 7. *Pheretima koryoensis*, n. sp. Showing the opening of testis sac to seminal vesicle, ca.  $\times 68$ . FS., ripen spermatozoa in funnel; MC., minute chambers.

the former is always of a single layer. The differentiation between these is distinctly recognized in vertical section. In the anterior pair, the posterior half of its epithelium is a derivative from the inner layer ("septal origin"), and its anterior half is a derivative from the outer layer ("parietal

*origin*"), or the vesicle itself is a sac formed by the branching of the doubled layer of the testis sac. This branching occurs near the portion of the posterior face of the septum X/XI. In the posterior pair, the vesicle itself is a sac formed by the direct branching of the doubled layer prolonged from the posterior face of septum XI/XII, where the doubled layer does not form the testis sac. So, the covering layer of the vesicle is always thinner than that of the testis sac. From this layer, very fine lamellae radiate inwards towards the ground mass of the vesicle, and by which the vesicle is inwardly subdivided into numerous minute chambers. The chambers are variable in size; perhaps that variation may be resulted by the sexual contents included within the chamber itself. The wall of the chamber is thickest in the region apparently incised and with connective tissue, perhaps its thickness may be also resulted by the contents of the sexual products.

The opening communicating the vesicle with the corresponding testis sac is large, or very large compared to that of *Lumbricus* (BUGNION and POPOFF) and to *Pheretima* sp. (BERGH); its diameter is about 0.5515 mm. in the septum X/XI and about 0.8205 mm. in XI/XII. In the region of the opening, there are not as many muscular fibres radiating in all directions as in BERGH's statement, though the fibres are slightly irregularly arranged, compared to the other regions. On the portion of the vesicle facing the testis sac, there is no actual covering layer, but slightly apart to it the fine series of the walls of the minute chambers are indistinctly arranged in a condition compressed posteriorly by the sexual products from the sac.

#### 4. Testes, Funnels, Sperm-ducts and Sexual products.

Testes and funnels are included within the testis sacs. The anterior testes are attached to the inner wall at about the middle of each limb of the anterior sac, slightly apart from the ventral blood vessel. The posterior testes are attached to the anterior inner wall of the sac, also slightly apart from the ventral blood vessel. The anterior testis is oval in shape, and has many processes in its free margin, so it is apparently much tufted, and measures about 0.8 by 1.4 mm. The posterior testis is somewhat discoidal and also apparently tufted, and measures about 1.5 mm. in diameter. The funnels are situated in the usual positions, and are of usual structure; they are rather large. The proximal portion of the testis is connected with the epithelium of the sac, where the muscular fibres of the latter are somewhat irregularly arranged, but never enter into the ground mass of the testis. Fine flattened epithelium derived from the

layer of the peritoneal cells of the sac, covers the testis as its investment, as has hitherto been studied in most earthworms. In the ground mass of the testis, any connective tissue was not found similarly as in *Lumbriculus variegatus* reported by R. HESSE (1894), except the blood vessels which are much supplied. Male cells in the testis are similarly developing in its every part. The sperm-duct arises from the narrowing end of the funnel, and after piercing the septum runs with slight windings caudalwards; ducts on a side unite at the posterior portion of the segment XIII; and are never sunk into the parietal wall. The male cells in the testis sac are slightly more advanced than those in the seminal vesicle; but no marked difference between them may be, in general, recognized, and each of them contains the male cells in all stages, distributed irregularly. The ventral nerve cord is situated just beneath (ventrally) the testis sacs.

*Pheretima* (Ph.) *fibula*, n. sp.

var. *typica*, n. var.

Five acitellate, and 19 clitellate specimens.

*Description of the type specimen* (September specimen).

*External characteristics:*

Length 95 mm., greatest diameter 6 mm., number of segments 107. Secondary annulations slightly developed on the segments anteroposterior to the clitellum. Colour, dorsally reddish brown and darker middorsally and anteriorly to the clitellum; clitellum dark brownish-grey.

Prostomium epilobous about  $\frac{1}{2}$ .

Setae beginning on II; setal ring without midventral break, and with a slight middorsal break posteriorly to the clitellum in the gradually decreasing width caudalwards, immediately posteriorly to the clitellum  $zz = 1\frac{1}{2} zy$ ; anteclitellar setae slightly smaller, especially the ventrolateral and lateral setae of the spermathecal segments smaller than the postoclitellar; ventral setae slightly more closely set than the dorsal; in general, no marked difference of the size between the dorsal and ventral setae distinguishable; approximate setal number as follows: 45/VI, 49/VII, 54/XX.

First dorsal pore in XII/XIII.

Clitellum annular, extending XIII/XIV-XVI/XVII, without setae, dorsal pores and intersegmental furrows.

Spermathecal pores, minute, 2 pairs anteriorly located on VI and VII, closely to the intersegmental furrows, about  $\frac{1}{4}$  of the circumference apart,

each pore simple opening, spermathecal setae about 25/VI, 27/VII.

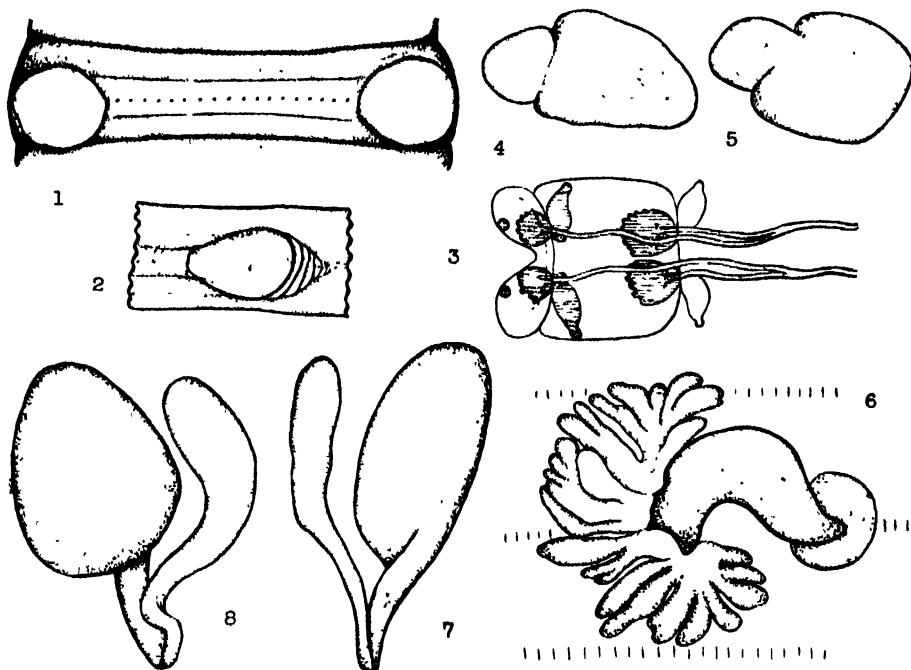
Female pore single, on XIV midventrally.

Male pores on the setal line of XVIII; each pore minute barely recognizable, at the centre of a papilla which is circular and centrally slightly elevated (somewhat conically protuberant), of about 1 mm. in diameter, its posterior side close to XVIII/XIX and anterior side about 1/3 of that segment apart from XVII/XVIII; pores about 1/4 of the circumference apart, separated by 18 setae (Fig. 8. 1).

The outer layer of the body wall of XVII and XIX in lines with the male pore-papillae slightly thickened and somewhat light-coloured, but the thickenings do not project into the coelom.

*Internal anatomy:*

Septum IV/V slightly thickened, V/VI-VII/VIII much thickened, VIII/IX ventrally present but vestigial, IX/X absent, X/XI and XI/XII much thickened, the succeeding septa gradually thinner.



Text-fig. 8. *Pheretima fibula*, var. *typica*, n. sp., n. var. 1. Ventral view of XVIII, ca.  $\times 10.4$ . 2. Ventrolateral view of XVIII found in two specimens, ca.  $\times 10.4$ . 3. Schematic showing of the ventral view of the anterior male organs. 4 and 5. Posterior view of seminal vesicles, 4-XII, 5-XI, ca.  $\times 16$ . 6. Prostate and flattened, nearly round glandular mass, ca.  $\times 10.4$ . 7 and 8. Spermathecae, 7-VI, 8-VII, ca.  $\times 10.4$ .

Gizzard rather small, bell-shaped and anteriorly narrower, smooth and shining on surface, extending about VII/VIII-X/XI. Intestine beginning to swell in XV. Intestinal coeca originated in XXVII, extending as far anteriorly as XXII, where they are bent under the intestine but long enough to reach into about XIX; each coecum on the ventral margin with about 10 serriformed, light-coloured outgrowths, on the dorsal margin with slight septal constrictions.

Nephridial tufts moderately thick, in V and VI, both septal and integumental nephridia rather conspicuous.

Commisural vessels, paired, those in IX incomplete, terminated dorsally on the posterior part of the gizzard, those in X asymmetrical, one loop much larger and regular in shape, but the other one rudimentary; hearts, 4 pairs in X-XIII, moderate in calibre, first two pairs enclosed within the corresponding testis sacs and slightly smaller than the rest; dorsal vessel small in calibre, but enlarged in the region of X-XIV; lymph glands moderately large, found on both sides of the dorsal vessel, behind middle portion of the body backwards.

Testis sacs annular, two in X and XI; closely related to those of *Ph. koryoensis*, though much smaller. Seminal vesicles, 2 pairs in XI and XII, each very small or vestigial, dorsoventrally flattened, with a very small primary ampulla, (at first glance, each vesicle somewhat resembles in appearance the body of *Fasciola hepatica* L.); each vesicle of the anterior pair antero-dorso-laterally within the testis sac; each vesicle of the posterior pair situated posteriorly to the ample testis sac and by the side of the gut, as a small hanging body of the sac, slightly larger than that of the anterior pair. Testes and funnels included within the sac, each testis of the anterior pair, disc-like, attached ventrally to the anterior inner wall of the sac, each funnel situated immediately posteriorly to the former; each testis of the posterior pair, somewhat oval, attached ventrally to the anterior inner wall of the sac, just beneath the sperm-duct of the anterior pair, each funnel situated posteriorly to the former, but not so closely as in the case of the anterior pair. Sperm-ducts on a side united with each other at the posterior part of XII. Pseudovesicles, one pair, attached to the posterior face of XII/XIII, very small, whitish, ovoid (Figs. 8. 3-5).

Prostate small; glandular portion occupying about XVII-XIX or XVIII (=2 or 3), lobular; duct stout, rather thick but short, forming a bow-shaped loop, decreasing in thickness ectally, such duct passing into a flattened, indistinct glandular thickening which is very slightly protruded into the coelom from the male pore-papilla (Fig. 8. 6).



Ovaries in XIII, usual in structure.

Spermathecae, 2 pairs in VI and VII; each ampulla ellipsoidal but slightly dorsoventrally flattened; duct moderately sharply marked off from the ampulla, stout, slightly shorter than the latter; diverticulum ectally passing into the anterior face of the duct, slightly shorter than the combined length of the duct and ampulla, ectal half somewhat tubular, ental half forming a moderately sized, elongated, oval widening (Figs. 8. 7 and 8).

. var. *ranunculus*, n. var.

Four acitellate and 13 clitellate specimens.

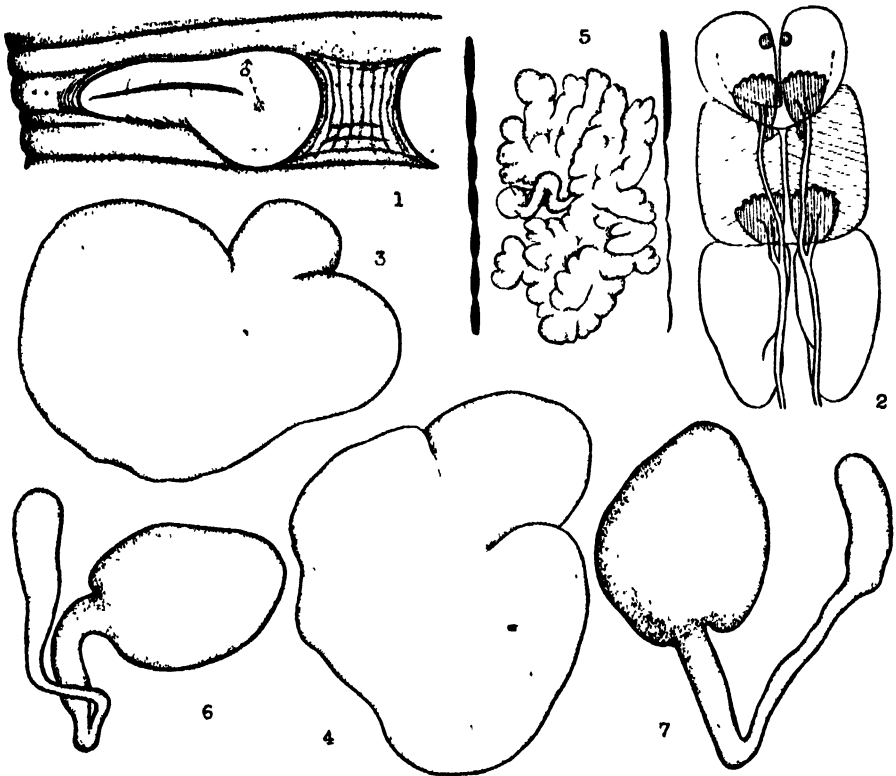
Externally, this variety, except for the male genital segment, closely resembles var. *typica* in all characteristics.

Male pores on the setal line of XVIII; each pore minute, barely recognizable, on a characteristic disc which somewhat resembles the tadpole laid with the head portion near to the ventral median line, its main part which resembles the head of tadpole, moderately protuberant, circular in outline, its posterior margin close to XVIII/XIX and anterior margin about 1/3 of XVIII apart from XVII/XVIII; the additional part which resembles the tail of the tadpole also moderately protuberant, but posterior and lateral sides slightly less, appearing to be laterally elongated on the setal zone from the main part; a male opening visible at the centre of the main part; diameter of the main part and the length of the additional part nearly equal to each other, about 2.7 mm.; the distance between two discs about 1.1 mm. on the setal line, where 7 setae are planted; on the surface of disc a distinct groove extends transversely from the lateral part near to the centre of the main part; setal number between male pore-discs varied 6-13, in most specimens 6 or 7, counted in only one 13 setae (Fig. 9. 1).

Internally, in most specimens prostate and anterior male organs differ from those of var. *typica*, but sometimes closely similar.

Testis sacs annular, two in X and XI; seminal vesicles mostly very much larger than those of var. *typica*, but in a few specimens small ones which are nearly equal to those of the latter in size, were found in XI and XII, anterior pair inclosed within the corresponding testis sac; they are related to those of *Ph. koryoensis* in structure. Anterior pair of seminal vesicles included, fully occupying the testis sac, dorsally and also ventrally closely meeting with each other, each vesicle with a moderately sized, smooth dorsal primary ampulla. Posterior pair of vesicles nearly equal to or slightly larger than the anterior pair, fully occupying that

segment, and more, strongly pushing XII/XIII posteriorly, each vesicle with a moderately sized, smooth dorsal primary ampulla which is larger than that of the anterior pair. But, the seminal vesicles show two types:



Text-fig. 9. *Pheretima fibula*, var. *ranunculus*, n. var. 1. Ventral view of XVIII, ca.  $\times 10.4$ . 2. Schematic showing of the ventral view of anterior male organs. 3 and 4. Posterior view of seminal vesicles, 3-XI, 4-XII, ca.  $\times 16$ . 5. Prostate (free hand drawing). 6 and 7. Spermathecae, 6-VI, 7-VII, ca.  $\times 10.4$ .

one is as above described, the other is quite similar to those of var. *typica*; out of 12 specimens opened, the former 10, the latter 2 found. So, by only these organs, both varieties are not separated from each other. Sperm-ducts on a side united at the anterior part of XII. Pseudovesicles similar in position and size to var. *typica* (Figs. 9. 2-4).

Prostate large, each gland occupying about XV or XVI-XXI (=6 or 7), consisting of about three main lobes, duct stout, rather long, entally pointing nearly straightly inwards and ectally forming a small horse-shoe-

shaped loop and then with its very slightly decreased thickness passing into a flattened small tadpole-shaped glandular thickening which is slightly protruded into the coelom of XVIII from the male pore-disc (its main circular part thicker and laterally elongated part less protuberant). In only one specimen out of 12 specimens opened, prostates closely similar to those of var. *typica* (Fig. 9. 5).

*Remarks :*

Variety *typica* :

In some July clitellate specimens, either setal pits or vestigial setae are ventrally found on each clitellar segment. Length varies between 82–95 mm., greatest diameter up to 6.5 mm., number of segments 80–113. In one specimen, a slight non-functional dorsal pore-like marking is visible in the intersegmental furrow XI/XII. A middorsal break in the setal ring, sometimes, may be indistinguishable. The approximate setal numbers of several specimens are indicated below :

II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XVII	XX	MP	XIV	XV	XVI	VI (SP)	VII (SP)	
28	36	46	44	50	52	55	54	50	55	55	53	58	60	16	8	11	13	26	26	*
26	33	41	41	45	49	49	46	47	45	47	51	56	53	18	0	0	0	25	27	**g
24	35	38	41	44	45	47	48	45	48	47	53	54	55	16	0	0	0	24	26	**
21	33	40	41	47	48	48	49	47	48	50	58	55	57	14	0	0	0	26	26	**
27	32	40	40	46	48	52	54	49	49	53	55	54	55	17	0	0	0	27	27	**

\* ..... July-specimen.

\*\* ..... September-specimen.

§ ..... Type specimen.

MP. .... Between male pore-discs.

VI (SP).... Between spermathecal pores.

In two specimens, the medial side of the male pore-papilla is very slightly elongated towards the midventral line, and its posterior margin not close to the intersegmental furrow, but slightly apart from it (Fig. 8. 2). The setal number between male pores varies from 14–18. The prostate glands are small, mostly occupying the segments from about XVIII–XIX, and sometimes XVII–XVIII or are only confined to the segment XVIII; in one specimen the gland is large and is formed quite similarly to that of var. *ranunculus*, extending from about XV–XXI.

Variety *ranunculus* :

Externally, the male pore-discs are characteristics and are formed almost similarly in all specimens at hand. But, the inner organs are variable and

perhaps may be actually more variable in many characteristics than those stated in the above description.

Considering from these, two varieties can be separated from each other, strictly saying, by a only characteristics, the appearance of the male genital segment. Perhaps, if a larger number of specimens of both forms are dissected, more close relation between the two may be detected.

The present species is readily distinguished from the other Korean species of the sub-genus by the appearance of the male genital segment, position of the spermathecal pore, seminal vesicles and spermathecae.

***Pheretima* (Ph.) *serrata*, n. sp.**

Three acitellate and 21 clitellate specimens.

*Description of the type specimen.*

*External characteristics:*

Length 98 mm., greatest diameter 5 mm., number of segments 114. Secondary annulations slightly developed on segments anteroposterior to clitellum. Colour, dorsally brown, darker middorsally and anteriorly to the clitellum; clitellum dark grey.

Prostomium epilobous about  $\frac{1}{2}$ .

Setae beginning on II; setal rings anterior to the clitellum and of end of the body on the slightly elevated ridges; setal ring with both middorsal and midventral breaks, in varied width caudalwards respectively, immediately posteriorly to the clitellum  $aa=1\frac{1}{2}-2$   $ab$  and  $zz=1\frac{1}{2}$   $zy$ ; ante-clitellar setae enlarged, especially distinct on III-VIII or IX; ventral setae slightly smaller and more closely set than the dorsal; approximate setal number as follows: 48/V, 52/VI, 56/VII, 68/XVII, 59/XX.

First dorsal pore in XII/XIII.

Clitellum entire, extending XIII/XIV-XVI/XVII, without setae, dorsal pores and intersegmental furrows.

Spermathecal pores, 3 pairs in V/VI, VI/VII and VII/VIII, each on a characteristic whitish, somewhat conical tiny tubercle, about  $\frac{2}{7}$  of the circumference apart, spermathecal setae 14/V, 15/VI, 15/VII (Fig. 10. 2).

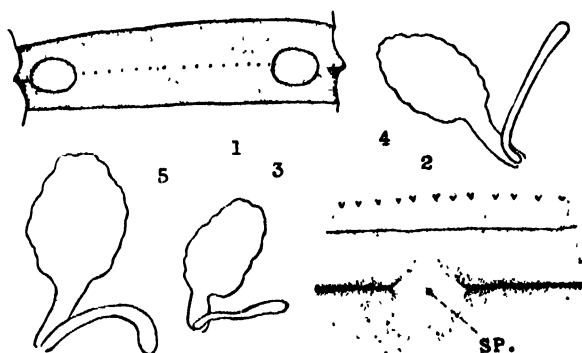
Male pores on the setal line of XVIII, minute and barely recognizable, each pore near the centre of a papilla which is situated on the setal line and is light-coloured, slightly transversely oval, slightly protuberant, of about 0.7 mm. in transverse length; pores about  $\frac{2}{9}$  of the circumference apart, 14 setae between them (Fig. 10. 1). Ventral outer layer of XVIII and XVII slightly thickened and light-coloured.

*Internal anatomy:*

Septum IV/V slightly thickened, V/VI-VII/VIII thickened, VIII/IX ventrally traceable, IX/X absent, X/XI-XII/XIII much thickened, XIII/XIV slightly thickened, the succeeding septa thin and membranous.

Pharyngeal glands not bulky, as usual.

Nephridial tufts moderately thick in V and VI, integumental ones rather distinct except gizzard segments, septal ones inconspicuous.



Text-fig. 10. *Pheretima serrata*, n. sp. 1. Ventral view of XVIII, ca.  $\times 15.6$ . 2. Spermathecal opening, ca.  $\times 26$ . 3, 4 and 5. Spermathecae, 3-VI, 4-VII; 5-VIII, ca.  $\times 15.6$ .

Gizzard moderate sized, barrel-shaped, occupying about VII/VIII-X/XI. Intestine beginning to swell in XV. Intestinal coeca originating in XXVII, extending as far anteriorly as XXIV, where they are bent under the intestine but long enough to reach into about XXII, each coecum on the ventral margin with about 10 serriformed, light-coloured outgrowths, on its anterior dorsal portion also with several similar but smaller appendages and posteriorly to that portion with slight septal constrictions.

Commisural vessels paired, those in IX incomplete, terminated dorsally on the posterior part of gizzard, those in X asymmetrical, one loop much larger and regular in shape, the other rudimentary; hearts, 4 pairs in X-XIII, first two pairs enclosed within the corresponding testis sacs, all moderate and nearly equal in calibre; dorsal vessel small in calibre, but enlarged in the region of X-XIV; lymph glands inconspicuous, but may be found on both sides of the dorsal vessel behind about XVIII/XIX backwards.

Testis sacs annular, two in X and XI; seminal vesicles, 2 pairs in XI and XII; they are closely related to those of *Ph. karyoensis*, but the

seminal vesicles are not as small as those generally found in *Ph. fibula typica*, each vesicle of XII without distinct dorsal primary ampulla and on its dorsal surface are found dark brownish dotted pigments (not usual). Sperm-ducts on a side united at the anterior part of XIII. Pseudovesicles, one pair, very small, attached to the posterior face of XII/XIII.

Ovaries moderate sized, in usual position.

Prostates large; each gland occupying about XVI-XXI, consisting of about 3 main lobes; duct relatively thin, its ectal about 1/3 vertically pointing and slightly thicker, its ental remaining thinner and nearly straight.

Spermathecae small, 3 pairs in VI, ~~VII~~ and VIII; each ampulla dorso-ventrally flattened oval, with slight peripheral zigzags but without indentations on the surface; duct shorter than the ampulla in length, not distinctly marked off from the latter; diverticulum slender, longer than the duct, but shorter than the combined length of the duct and ampulla, ectally passing into the anterior face of the duct, entally bent towards the gut and no marked widening formed at its terminal.

*Remarks:*

The specimens with incomplete clitellar glandularity are mostly with slight intersegmental furrows, setal pits, and seldom with non-functional dorsal pore-like markings on the clitellum. Length varies from 74-109 mm., greatest diameter up to 5.5 mm., number of segments 101-117. The middorsal break in the setal ring is always visible, but midventral one, sometimes may be indistinguishable. The approximate setal number of a few specimens are indicated below:

II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XVII	XX	XXX	MP	V (SP)	VI (SP)	VII (SP)
28	31	38	43	48	52	53	53	54	60	59	62	60	59	57	12	15	15	16
28	35	39	48	52	56	56	60	55	58	61	61	68	59	61	14	15	15	15
28	34	39	42	46	53	54	53	52	54	52	58	62	64	65	14	14	14	15
26	33	40	42	46	51	55	56	59	59	58	60	63	65	63	14	14	14	15

V (SP)....between spermathecal pores on V.

MP .....between male pores.

Setal number. between male pores is varied in range of 12-14. In all specimens, first dorsal pore was found in the intersegmental furrow XII/XIII. Papillae bearing the male pores are, in most cases, oval in shape, sometimes, nearly round. On the anterior part of the dorsal margin of the

coecum, the outgrowths, sometimes may be found. Prostatic duct variable in shape; mostly with a somewhat vertical and horse-shoe-shaped loop, and sometimes it is once coiled at its middle portion forming an O, but its ectal half is always slightly thicker than the ental.

Peripheral zigzags of the spermathecal ampulla are not so marked as in *Ph. kamitai*, *Ph. maculosus* and *Ph. phaselus*, and sometimes may be scarcely recognizable. Of these three species, the present species more closely resembles the first, but can be easily separated from it not only in the spermathecae, but also in the setal size, appearance of the spermathecal pores, intestinal coeca, prostates, and especially in the anterior male organs.

*Pheretima (Ph.) monstrifera*, n. sp.

One clitellate specimen. (it was also found from other localities, one clitellate specimen, Bunkei (聞慶), Keishô-hoku-dô, by Mr. SHIN, August, 1934; two clitellate specimens, Zuizan (瑞山), Chûsei-nan-dô, Mr. Ryû, August, 1934; one clitellate specimen, Heiten (餅店), Keiki-dô, Mr. Ri, August, 1934).

*Description.*

*External characteristics.*

Length 235 mm., greatest diameter 8.5 mm., number of segments 137. Secondary annulations, on IV-IX with 3 annuli. Colour, dorsally dark reddish brown, being marked anteriorly to the clitellum, ventrally yellowish grey; clitellum dark brownish grey.

Prostomium epilobous about  $\frac{1}{2}$ .

Setae beginning on II; setal ring with both middorsal and midventral breaks in varied width,  $aa = 1\frac{1}{2}-2\ ab$ ,  $zz = 1\frac{1}{2}-2\ zy$ ; clitellum without setae; on II-male pore region enlarged, in which, II-IX markedly, X-XIII slightly, male pore region moderate; on all segments through the body some (about  $a-e$ ) midventral setae slightly enlarged and in which  $a > b > c > d > e$  .... in a very small degree, and then from laterad to dorsum gradually enlarged again in a similar manner; on II-male pore region ventral setae larger than the dorsal, and from this portion to about XXXV no marked difference of the size between them noticed, and posteriorly to this the former are rather smaller than the latter; on II-male pore region setal interval ventrally and dorsally may be nearly equal, but posteriorly to this they are ventrally more closely set than dorsally; approximate setal number are as follows: 21/II, 23/III, 28/IV, 29/V, 32/VI, 38/VII, 43/VIII,

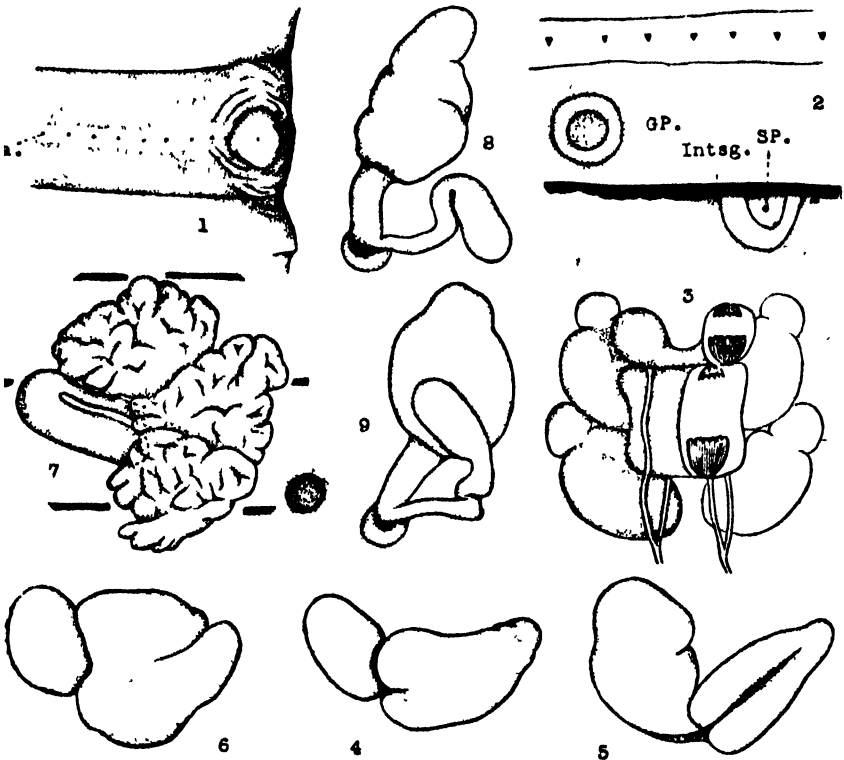
# EARTHWORMS FROM KÔRYÔ, KOREA

45/IX, 55/X, 63/XI, 64/XII, 68/XIII, 66/XVII, 73/XX, 73/XXX, 67—middle portion of the body, 14—between male pores, spermathecal setae 11/V, 13/VI, 13/VII, 14/VIII.

First dorsal pore in XII/XIII.

Clitellum entire, extending XIII/XIV–XVI/XVII, without setae, dorsal pores and intersegmental furrows.

Spermathecal pores, 4 pairs, anteriorly located on VI, VII, VIII and IX, quite close to the intersegmental furrows, about  $\frac{1}{3}$  of the circumference apart, each pore on a minute greyish tubercle (apparently whitish spot), and surrounding each pore and just posteriorly to the intersegmental furrow a half-ring-like papilla placed, about 0.8 mm. in diameter (Fig. 11. 2).



Text-fig. 11. *Pheretima monstrifera*, n. sp. 1. Ventral view of XVIII, ca.  $\times 10.4$ . 2. Ventral view of a side of VIII and IX, ca.  $\times 16$ ; GP., genital papilla; SP., Spermathecal pore; Intsg., intersegmental furrow VIII/IX. 3. Schematic showing of the ventral view of anterior male organs. 4, 5 and 6. Showing the variation of seminal vesicle of XII (posterior view), ca.  $\times 10.4$ . 7. Prostate, ca.  $\times 6.5$ . 8 and 9. Spermathecae, 8–VIII, 9–IX, ca.  $\times 10.4$ .



Female pore single, midventrally on XIV.

Male pores ventrolaterally on the setal line of XVIII, each pore at the centre of a rather small, poorly demarcated but nearly circular, slightly protuberant, greyish papilla with one or more incomplete circumferential furrows; pores about  $3/8$  of the circumference apart, and between them 14 setae present (Fig. 11. 1).

Genital papillae, one pair on VIII, behind the setal line, slightly medial to the spermathecal line, each papilla circular, centrally slightly depressed, of nearly equal diameter to the spermathecal papilla, between two papillae 8 setae, and on each side between spermathecal and genital papillae about 3 setae present (Fig. 11. 2).

*Internal anatomy:*

Septum IV/V slightly thickened, V/VI-VII/VIII much thickened, VIII/IX ventrally traceable, IX/X absent, X/XI and XI/XII very much thickened, XII/XIII much thickened, the succeeding septa gradually thinner.

Nephridial masses, thick in V and VI.

Gizzard, of moderate size, somewhat bell-shaped and posteriorly broader. Intestine beginning to swell in XV. Intestinal coeca finger-shaped, originating in XXVII, extending as far anteriorly as XXIII, where they are bent towards the ventral side, but long enough to reach into XXII; each coecum on the dorsal margin with several light-coloured indistinct, appendages, on the dorsal margin with some similar but smaller ones.

Commisural vessels, paired, those in IX, incomplete and terminated dorsally on the posterior part of gizzard, those in X, asymmetrical, one loop much larger and regular in shape, the other rudimentary; hearts, 4 pairs in X-XIII, all large in calibre, the first two smaller than the others; dorsal vessel large in calibre, and much enlarged in the region of X-XIV or XV; lymph glands not found.

Testis sacs in X and XI, each a single ventral median sac separated by the septum from each other; anterior one smaller, with rounded antero-lateral limbs, posterior one larger, ventrally extending from septum to septum, somewhat quadrate in shape. Seminal vesicles, 2 pairs in XI and XII, each rather small compared to body size; anterior pair slightly smaller than the posterior, each vesicle rounded and with a rounded smooth large dorsal primary ampulla which is slightly sunk into the vesicle body; posterior pair characteristic in shape, each vesicle flattened and rather leaf-like, and with a relatively very large rounded smooth dorsal primary ampulla which is markedly constricted from the body (but its form may be variable to some extent, as shown in the figures 4-6).

Testes and funnels in usual position. Sperm-ducts on a side united at the middle part of XII. Pseudovesicles, 2 pairs, each of them moderately large, attached to the posterior faces of XII/XIII and XIII/XIV respectively (Fig. 11. 3-6).

Ovaries large and in usual position.

Prostates small; each gland occupying about XVII and XVIII, consisting of 3 main lobes; duct stout, shining, with a somewhat hair-pin-shaped loop, its ectal half very much thicker (Fig. 11. 7).

Spermathecae, 4 pairs in VI, VII, VIII and IX; each ampulla slightly dorsoventrally flattened, elongated oval, those of VIII and IX broader than the others; duct rather thick, stout, distinctly marked off from the ampulla, slightly shorter than the latter in length; diverticulum ectally passing into the anterior face of the duct, slightly thinner but longer than the latter and slightly shorter than the combined length of duct and ampulla, its ectal about  $\frac{3}{5}$  nearly straight (in VI and VII) or slightly curved (in VIII and IX), its ental remaining forms a large elongated oval widening (Fig. 11. 8 and 9).

Corresponding to the external spermathecal papilla, a half-ring-like whitish glandular mass indistinctly protrudes into the coelom surrounding the ectal-most portion of the spermathecal duct. That glandular mass is anteriorly gradually less conspicuous, such as in VI it may be scarcely recognized. Slightly more ventrolaterally to the spermatheca of IX a whitish poorly demarcated circular glandular mass with roughened surface is very slightly protruded into the coelom.

*Remarks :*

The present species is easily distinguished from the other Korean species of the sub-genus, by the large size of the body, genital papillae on VIII, setal arrangement, and anterior and posterior male organs.

*Pheretima* (Ph.) *susakii*, n. sp.

var. *typica*, n. var.

Fifteen acitellate and 37 clitellate specimens.

*Description.*

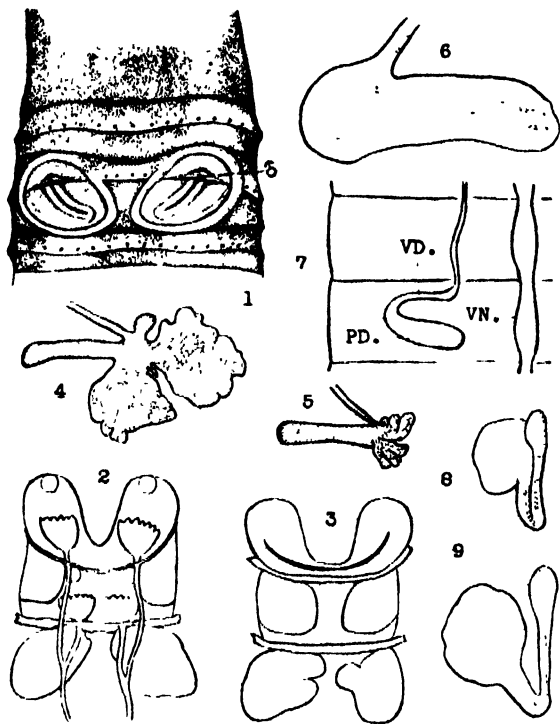
*External characteristics :*

Length 44-64 mm., greatest diameter up to 3 mm., number of segments 94-106. Secondary annulations slightly developed anteroposteriorly to the clitellum, V-X and about XVII-XXXV with 3 annuli. Colour, dorsally yellowish brown, lighter anteriorly and rather darker immediately posteriorly

to the clitellum; clitellum dark brown or dark reddish brown.

Prostomium epilobous about  $\frac{1}{2}$ .

Setae large relatively to the body size and beginning on II; setal ring without midventral break, very slight if present, and with slight middorsal break,  $zz=1 \frac{1}{3}$ –2  $zy$  (sometimes, it may be indistinguishable); on II–male



Text-fig. 12. *Pheretima susakii*, var. *typica*, n. sp., n. var.  
1. Ventral view of XVII, XVIII and XIX, ca.  $\times 15.6$ . 2 and 3. Schematic showings of the anterior male organs, 2–ventral view, 3–dorsal view. 4 and 5. Prostates taken from one specimen, 4 from left-side, 5 from right-side, ca.  $\times 15.6$ . 6 and 7. Prostates found in most specimens, 6–cleared, ca.  $\times 39$ , 7–schematic showing of the former, VD–vas deferens, PD–prostatic duct, VN–ventral nerve cord. 8 and 9 Spermathecae, 8–VII, 9–VIII, ca.  $\times 15.6$ .

pore region (clitellum without setae) and on several segments of the end of the body enlarged, in which in the latter case they are planted on the raised ridges; ventral setae more closely set than the dorsal, and the former very slightly larger, especially on male pore region distinctly larger

than the latter, sometimes posteriorly to this region no marked difference of the size between them may be noticed. Approximate setal numbers of a few specimens are indicated below :

II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XVI	XX	XXX	MP	SP	
																VI/V/D	VII/V/D
26	31	33	38	39	39	40	39	38	41	40	43	43	45	43	4	24/15	24/16
27	32	33	38	42	42	41	41	42	42	43	43	46	46	44	6	27/15	27/15
26	32	32	38	41	40	40	41	42	42	40	42	45	44	43	4	26/15	25/15
28	31	33	37	40	41	41	41	42	43	42	43	45	46	43	4	25/15	26/15

MP....between male pores.

SP. VI/V/D....(V....ventral, D....dorsal), setal numbers on both ventral and dorsal surfaces, counted separately in lines with the spermathecal pores.

Functional first dorsal pore, mostly in XIII/XIV, sometimes in XII/XIII and more frequently the non-functional dorsal pore-like marking is found in XII/XIII.

Clitellum entire, extending XIII/XIV–XVI/XVII, without setae, dorsal pores and intersegmental furrows. Sometimes, its ventral portion is slightly concave, and ventral clitellar glandularity slightly extending anteriorly and posteriorly over intersegmental furrows XIII/XIV and XVI/XVII.

Spermathecal pores, 2 pairs in VI/VII and VII/VIII, each pore relatively large on a tiny tubercle which is always situated on the intersegmental furrow; about 7/11 of the circumference apart, spermathecal setae varied in range of 23–27.

Male pores ventrally on the setal line of XVIII; each pore on a characteristic genital area which is not so protuberant in general appearance, somewhat oval, diagonally situated with posterior end to be nearer to the midventral line and to close to that of the opposite one, its posterior and anterior margins quite close to the intersegmental furrows or by the areas these furrows are anteriorly and posteriorly displaced, so XVIII actually much longer than the rest; each area distinctly delimited by a glandular, light-coloured, slightly elevated circumferential band, and on such a circumvallated area a rather distinct but narrow groove diagonally running along its long axis; that groove fringed with bank-like elevated glandular band, in most cases, which is cut by the slight depression formed in the seta line; in the anterior end of that depression male opening situated; the other portion on the surface of the area also glandulated, light-coloured but less elevated; sometimes the region between the areas

also becoming glandular, light-coloured and very slightly elevated ; positions of the male pores about 3/13 of the circumference apart, between them 4-6 setae planted (4 setae-11 specimens, 5-7 specimens, 6-6 specimens, in 24 specimens examined). In most specimens, the outer layers of XVII and XIX on which the regions in lines with the male genital areas are slightly thickened and light-coloured (Fig. 12. 1).

*Internal anatomy :*

Septa generally thin ; IV/V and V/Vi membranous, VI/VII and VII/VIII slightly thickened, VIII/IX and IX/X absent, X/XI and XI/XII slightly thickened, the succeeding septa thin and membranous.

Pharyngeal glands moderately bulky.

Nephridial tufts moderately thick, both septal and integumental ones inconspicuous. Gizzard large compared to body size, barrel-shaped and anteriorly narrower, nearly smooth and shining on surface, occupying about VII/VIII-X/XI. Intestine beginning to swell in XV. Intestinal coeca simple, finger-shaped, with slight septal constrictions dorsally, originating in XXVII, extending as far anteriorly as XXIV or XXIII, the anterior portion of each coecum slightly bent towards the ventral side.

Commissural vessels, paired, very small in size, those in IX, incomplete and terminate dorsally on the posterior part of gizzard, those in X asymmetrical, one loop larger, the other one smaller or slightly smaller ; hearts, 4 pairs in X-XIII, first two pairs enclosed within the testis sacs, all rather small in calibre ; dorsal vessel moderate in calibre, enlarged in the region of X-XIV ; lymph glands found on both sides of the dorsal vessel behind coecal segment backwards.

Testis sacs annular, two in X and XI ; seminal vesicles, 2 pairs in XI and XII ; their relationship closely resembles that in *Ph. koryoensis*, though each organ much smaller than that of the latter ; each seminal vesicle of the anterior pair is a rounded body with indistinct apical dorsal primary ampulla, each of the posterior pair ovoidal body with a large dorsal primary ampulla, each ampulla smooth and each vesicle slightly vesicular on surface. Sperm-ducts on a side united at the anterior or middle portion of XII. Pseudovesicles not found (Figs. 12. 2 and 3).

Ovaries large compared to size of the body, situated on usual position.

Prostates rudimentary ; glandular portion absent and represented only as a very small, short, club-shaped duct in XVIII ; sometimes, rudimentary glandular portion may be found as in figures (Figs. 12. 4-7).

Spermathecae, 2 pairs in VII and VIII, large compared to body size ; each ampulla slightly dorsoventrally flattened, smooth on surface, that of

VIII larger and round, that of VII smaller and oval in shape; duct relatively thick, stout, shining on surface, not sharply marked off from the latter, slightly shorter than the ampulla in length; diverticulum nearly equal to or slightly shorter than the combined length of the duct and ampulla, nearly straight, tubular, and at its entalmost portion forming a small round or elongated oval widening (Figs. 12. 8 and 9).

*Remarks:*

The present species is the smallest form of the sub-genus that has been collected in Korea, and even only by that characteristic it is easily distinguished from the other Korean species.

var. *patina*, n. var.

Sixteen acitellate and 28 clitellate specimens.

The present variety differs from var. *typica* in the following characters: (1) a little smaller size of the body, (2) presence or distinctness of the midventral break in the setal ring, (3) male genital area, (4) size and shape of the seminal vesicles, (5) glandular portion of the prostate.

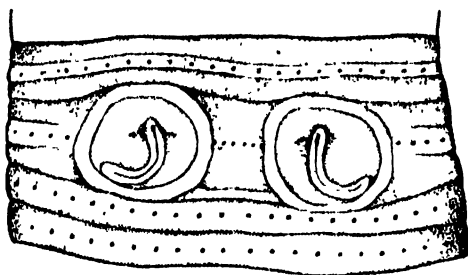
In the other characteristics it is closely similar to the latter.

(1) Length 33-50 mm., greatest diameter up to 3 mm., number of segments 99-102.

(2) Setal ring with both midventral and middorsal breaks, and each in gradually decreasing width caudalwards, those in the region immediately posterior to clitellum  $aa=1\frac{1}{2}$ -2  $ab$ ,  $zz=1\frac{2}{3}$ -2  $1\frac{1}{3}$   $zy$ .

(3) Male pores ventrally on the setal line of XVIII; each pore on a characteristic genital area which is not so protuberant in general appearance, nearly circular in outline, both medial sides close to ventral median line, its posterior and anterior margins quite close to the inter-segmental furrows or rather to the area XVII/XVIII and XVIII/XIX are anteriorly and posteriorly displaced, so XVIII actually much longer than the neighbouring segments; each area distinctly delimited by a glandular, light-coloured, slightly elevated, circumferential band, on such a circumvallated area a rather distinct but narrow groove running in a C-shape laterally faced, the groove fringed with the bank-like elevated glandular band, in most cases which is cut by the slight depression formed faintly in the setal line; in the anterior end of the groove a male pore opened; positions of the male pores about  $\frac{1}{4}$  of the circumference apart, between them 4-6 setae are planted (4 setae-2 specimens, 5-6 specimens, 6-18 specimens, in 25 specimens examined). In most specimens, the outer

layer of XVII and XIX in line with the male genital area is slightly thickened and light coloured (Fig. 13).



Text-fig. 13. *Pheretima susabii*, var. *patina*, n. var. Ventral view of XVIII. ca.  $\times 20$ .

(4) Seminal vesicles, 2 pairs in XI and XII, anterior pair enclosed in the corresponding testis sac. Those of the anterior pair much smaller than the posterior, each with a moderate sized smooth and apparently slightly shining dorsal primary ampulla; each of the

posterior pair with a large, smooth or slightly vesicular dorsal primary ampulla; each vesicle body vesicular on surface.

(5) Prostate moderate or rather large; glandular portion extending XVII-XX or sometimes XVI-XXII.

### *Pheretima* (Ph.) *vallis*, n. sp.

Three acitellate and 2 clitellate specimens.

#### *Description.*

#### *External characteristics:*

Length 210 (180) mm., greatest diameter 10.5 mm., number of segments 134 (135). Secondary annulations, V or VI-XIII and XVII-XIX or XX with 3 annuli. Colour, dorsally dark brown, concentrated anteriorly to the clitellum, ventrally yellowish grey; clitellum dark grey.

Prostomium epilobous, about  $\frac{1}{2}$ .

Setae beginning on II, and rather small; setal ring almost completely close, or very slight if present; antecitellar setae slightly larger than the postocitellar, but in the former the ventral setae of the spermathecal segments are much shortened and delicate, thus it sometimes becomes difficult to count their number; generally, dorsal setae slightly larger than the ventral; no marked difference of the setal interval between them distinguishable; approximate setal numbers are indicated below:

First dorsal pore in XII/XIII.

Clitellum entire, extending XIII/XIV-XVI/XVII, without setae, dorsal pores and intersegmental furrows.

Spermathecal pores, 3 pairs, anteriorly located on VI, VII and VIII,

III	VI	VII	VIII	X	XIII	XVII	XX	XXX	MB	MP	SP		
											VI/V/D	VII/V/D	VIII/V/D
42	68	74	79	76	82	80	92	88	82	9	45/23	50/23	50/29
44	69	71	75	74	78	79	88	88	84	10	45/24	45/26	46/29

MB....on the middle portion of the body.

MP....between male pores.

SP. VI/V/D....(V-ventral, D-dorsal), setal numbers on both surfaces, counted separately in lines with the spermathecal pores.

quite close to the intersegmental furrows, dorsolaterally, about  $3/5$  of the circumference apart; each pore minute and on a light-coloured, slight thickening of the outer layer of the body wall.

Male pores ventrolaterally on the setal line of XVIII; each pore on a characteristic genital area which is light-coloured, poorly delimited but may be said to be of an oval, slightly diagonal to be nearer with the anterior end to midventral line, its posterior margin close to XVIII/XIX and the anterior end does not reach to XVII/XVIII being apart from about  $1/5$  of that segment or occupying 2 annuli (XVIII with 3 annuli), transversely about 11 setal wide; on such an area along the long axis extends a relatively deep groove with its bank-like elevated glandular band, that groove is distinct on the third annulus, indistinct on the second annulus; its anterior end corresponding to the male opening situated on the setal line which is also slightly glandulated, light-coloured and elevated; positions of the male pores about  $1/5$  of the circumference apart, between the areas 9 (10) setae present (Fig. 14. 1).

#### *Internal anatomy:*

Septum IV/V slightly thickened, V/VI and VI/VII much thickened, VII/VIII very much thickened, VIII/IX ventrally traceable, IX/X absent, X/XI and XI/XII very much thickened, the succeeding septa gradually thinner.

Pharyngeal glands rather bulky.

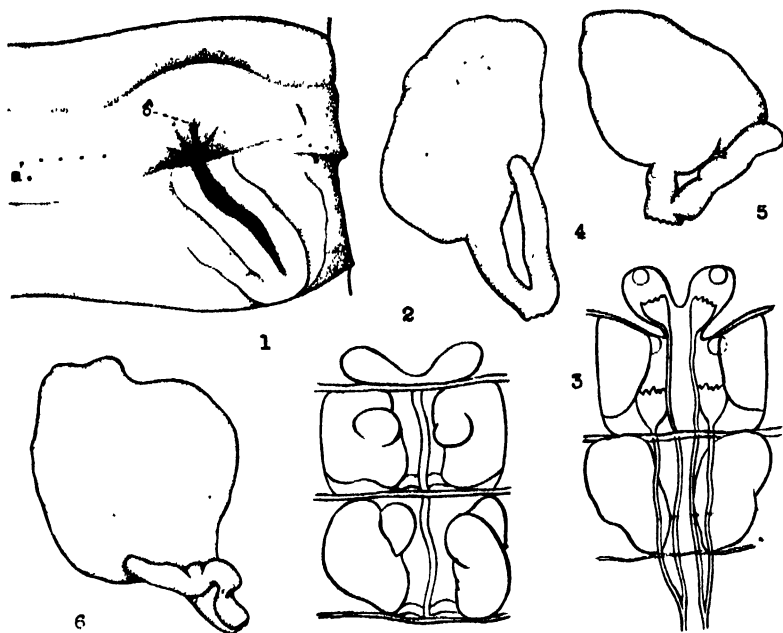
Nephridial tufts moderately thick in V and VI, both septal and integumental inconspicuous.

Gizzard large, bell-shaped and anteriorly narrower, extending about VII/VIII-X/XI, vascular on surface. Intestine beginning to swell in XV. Intestinal coeca finger-shaped, originating in XXVII, extending as far anteriorly as XXIV, where they are bent under the intestine but long enough to reach into XXI; each coecum on the posterior half of the



ventral margin with several serriformed and light-coloured appendages, on the dorsal margin with slight septal constrictions.

Testis sacs in X and XI; they are annular and similar to those of *Ph. koryoensis*, but in some points differ from the latter; two annular sacs communicating with each other by a midventral bridge; anterior sac includes ventral nerve cord as well as testes, funnels, hearts, dorsal and ventral blood vessels and sexual products, posterior sac includes also those besides others, seminal vesicles of that segment and sperm-ducts of the



Text-fig. 14. *Pheretima vallis*, n. sp. 1. Ventral view of XVIII, ca.  $\times 10.4$ . 2 and 3. Schematic showings of the anterior male organs, 2-dorsal view, 3-ventral view. 4, 5 and 6. Spermathecae, 4-VI, 5-VII, 6-VIII, ca.  $\times 10.4$ .

anterior pair. Seminal vesicles relatively small; both vesicles in each segment not meeting in the middorsal line; anterior pair slightly smaller than the posterior; each vesicle nearly smooth on surface and with a moderate sized smooth dorsal primary ampulla. Testes and funnels large, situated in usual position, the former apparently tufted. Sperm-ducts on a side united at the middle portion of XIII. Pseudovesicles, one pair, attached to the posterior face of XII/XIII, very small, sometimes rudimentary or may not be found (Fig. 14. 2 and 3).

Commissural vessels, paired, those in IX incomplete, terminated dorsally

on the posterior part of the gizzard, those in X asymmetrical, one loop much larger and regular in shape, the other rudimentary; dorsal vessel large in calibre and enlarged in the region of X-XIV; hearts, 4 pairs in X-XIII, large in calibre, but first two pairs smaller than the rest and enclosed within the corresponding testis sacs; lymph glands not found.

Ovaries large and in usual position.

Prostates very small, only confined to XVIII; each gland very small, consisting of about 4 or 5 lobes; duct straight, short but longer than the transverse length of the glandular portion, thin but stout and shining, with very fine supporting tissue, ectally gradually thicker in a very slight degree; such duct passing into a poorly formed, flattened, whitish glandular mass which very slightly protrudes into the coelom from the external male genital area, sometimes it may be indistinct; sperm-duct rather thick united at the proximal end, of the duct.

Spermathecae moderate sized, 3 pairs; each ampulla dorsoventrally flattened, pear-shaped; duct short in comparison to large ampulla, less than  $\frac{1}{2}$  of the latter in length, but with moderate thickness, indistinctly marked off from the latter; diverticulum ectally passing into the anterior face of the duct, short but rather thick and slightly thinner but longer than the duct, nearly equal to  $\frac{1}{2}$  of the combined length of the duct and ampulla, nearly straight or slightly twisted, entally forming no marked widening (Fig. 14. 4-6).

*Remarks:*

The present species is much larger than the other members of Korean forms of the sub-genus, even in this point it is easy to distinguish the present one from them, and it is also different from *Ph. monstifera* in many characteristics. It stands as a distinct species characterized by the male genital area, anterior and posterior male organs, spermathecae and positions of the spermathecal pores.

***Pheretima (Ph.) bitheca*, n. sp.**

Eleven clitellate specimens.

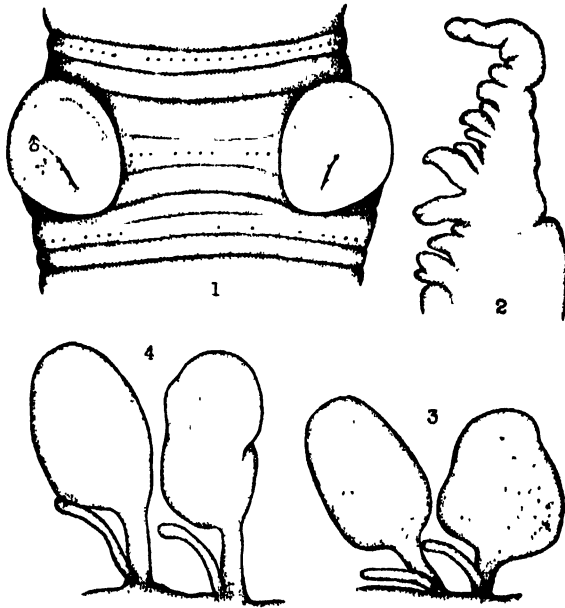
*Description of the type specimen.*

*External characteristics:*

Length 88 mm., greatest diameter 5 mm., number of segment 114. Secondary annulations slightly developed on the segments anteroposterior to the clitellum. Colour, dorsally reddish brown, darker middorsally and anteriorly to the clitellum; clitellum dark brownish grey.

Prostomium epilobous about  $\frac{1}{2}$ .

Setae beginning on II; setal ring without midventral break and with a very slight middorsal break posteriorly to the clitellum in the gradually decreasing width caudalwards, on the posterior end of the body it may be indistinguishable; antecitellar setae may be slightly smaller than the postoclitellar, or both of them may be nearly equal in size; ventrolateral and lateral setae of the spermathecal segments smaller than the rest; ventral setae slightly more closely set than the dorsal, no marked difference distinguishable in size between the ventral and dorsal setae; approximate setal number as follows: 52/VI, 59/VII, 62/XX.



Text-fig. 15. *Pheretima bitheca*, n. sp. 1. Ventral view of XVII, XVIII and XIX, ca.  $\times 15.6$ . 2. Intestinal coeca, ca.  $\times 15.6$ . 3 and 4. Spermathecae in each group, 3-VI, 4-VII, ca.  $\times 15.6$ .

First dorsal pore in XII/XIII. (In 4 specimens non-functional dorsal pore-like marking was found in XI/XII).

Spermathecal pores minute, each with a slight epidermal elevation; transversely two grouped pairs anteriorly located on VI and VII, quite close to the intersegmental furrows; each group consisting of 2 by 2 pores which are separated from each other by 2 or 3 setae; each inner (more ventral) pair about  $\frac{1}{2}$  of the circumference apart, and setal number between

them 27/VI and 32/VII, respectively.

Female pore single, on XIV midventrally.

Male pores ventrally on the setal line of XVIII; each pore minute, on a characteristic disc which is somewhat oval and diagonally placed with the posterior end nearer to the midventral line, its posterior and anterior ends close to XVII/XVIII and XVIII/XIX respectively, antero-laterally markedly and posteromedially less protuberant, on its surface a rather distinct but short groove extends diagonally (along its long axis) from near the centre where the male opening situated, to the postero-medial side (but it never reaches the posterior end of the disc); positions of the male pores about  $1/4$  of the circumference apart; between discs 14 setae planted on (Fig. 15. 1).

*Internal anatomy;*

Septum IV/V slightly thickened, V/VI-VII/VIII thickened, VIII/IX and IX/X absent, X/XI and XI/XII much thickened, XII/XIII thickened, the succeeding septa gradually thinner.

Pharyngeal glands not bulky, as usual.

Nephridial tufts not so thick, both septal and integumental ones rather distinct.

Commissural vessels, paired, those in IX incomplete and terminated dorsally on the posterior part of the gizzard, those in X asymmetrical, one loop large, the other rudimentary; dorsal vessel rather small in calibre, slightly enlarged in the region of X-XIV; hearts, 4 pairs in X-XIII, rather small in calibre, first two pairs enclosed within the corresponding testis sacs; lymph glands rather distinct, found on both sides of the dorsal vessel behind the middle portion of the body caudalwards.

Gizzard moderate sized or small, bell- or barrel-shaped and anteriorly slightly narrower, smooth and shining on surface, occupying about VII/VIII-X/XI. Intestine beginning to swell in XV. Intestinal coeca originating in XXVII, extending as far anteriorly as about XXII, where they are bent under the intestine, but long enough to reach into about XX; each coecum on the ventral margin with about 10 serriformed, light-coloured appendages (Fig. 15. 2).

Testis sacs annular, two in X and XI; seminal vesicles, 2 pairs in XI and XII; these anterior male organs closely resemble those which most frequently occur in *Ph. fibula typica*. Sperm-ducts on a side united at the middle part of XII. Pseudovesicles, one pair, very small, attached to the posterior face of XII/XIII.

Ovaries in usual position.

Prostates small; each gland small, lobular, occupying about XVII and XVIII; duct stout, with a V-shaped loop, its ectalmost portion slightly thinner and passing into a flattened indistinct glandular mass (from the male pore disc).

Spermathecae small, in VI and VII, grouped and transversely arranged in two by two; each ampulla somewhat ellipsoidal but slightly dorsoventrally flattened; duct about  $\frac{1}{2}$  of the ampulla in length, not sharply marked off from the latter; diverticulum slender, either slightly shorter or longer than the duct, no marked widening at its terminal (Figs. 15. 3 and 4).

**Remarks:**

Length varies from 85–98 mm., greatest diameter up to 6.5 mm., number of segments 104–114. A middorsal break in the setal ring, sometimes may be indistinguishable. The approximate setal numbers of a few specimens are indicated below:

II	III	IV	V	VI	VII	VIII	XI	X	XI	XII	XIII	XVII	XX	XXX	MP	SP (VI)	SP (VII)
28	38	46	48	52	59	58	56	60	60	62	60	62	62	51	14	27	32*
26	36	42	45	50	56	57	60	56	57	63	62	64	60	54	14	28	30
22	35	44	47	53	58	60	57	57	64	65	61	62	56	50	15	27	29
27	32	40	48	55	55	61	59	56	53	62	61	58	61	58	14	27	27

\* .....type specimen.

MP.....between male pore discs.

SP (VI)....between the inner (more ventral) pair of the spermathecal pores.

The functional first dorsal pore was found in XII/XIII. Spermathecal pores are so minute, that it is difficult to detect them, but their situations may be found indirectly from the slight epidermal elevations surrounding the pores; without exception the openings and spermathecae are transversely in two grouped pairs, each group consists of two by two pores; this is the most distinct characteristics for the present species. Externally, the male pore discs are also characteristic; in all specimens they are formed as similarly as in the description and figure of the type specimen. In all specimens opened, the anterior male organs closely resemble those most frequently found in *Ph. fibula typica*. Prostates are small, sometimes are only confined to XVIII.

The present species is somewhat related to the species of sub-genus *Polypheretima* MICHAELSEN (1934), and more closely related to *Ph. bleck-*

*wenni* UDE in the presence of the well-developed intestinal coeca, but is readily distinguishable from that in body size and colouration, male pore discs and setal number between them, setal arrangement, spermathecae, and especially in the anterior male organs.

### Family LUMBRICIDAE

Genus *EISENIA* Malm em. MICHAELSEN

*Eisenia rosea* (SAVIGNY)

Several acitellate and clitellate specimens.

Genus *ALLOLOBOPHORA* Eisen em. ROSA

*Allolobophora japonica* MICHAELSEN?

Two clitellate specimens.

This worm is identical with MICHAELSEN's species in all except two characteristics, setal interval and absence of papillae bearing the setae. The setal interval is measured on XX,  $aa:bc=39:21$ , so it is quite different from that of MICHAELSEN's species; and in two specimens no papillae were found on any segments. (And, except these two differences, it is identical with the smaller type according to BEDDARD's remarks, and with the subsp. *typica* according to OISHI's remarks).

Genus *BIMASTUS* MOORE

*Bimastus* sp.

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# THE ENDOSPERM FORMATION IN *CRYPTOMERIA JAPONICA*

By

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(With twenty-two figures)

(Received April 25, 1936)

In 1904 LAWSON gave an account concerning the gametophyte of *Cryptomeria japonica*. According to him, the wall formation in the endosperm development of this plant is quite peculiar, a similar case being so far reported in no other plant. The present study was primarily undertaken to ascertain the correctness of his description. The material was obtained mainly from trees growing in the garden of our University at Sendai. The collections were made almost daily, from February to July. Chromo-acetic acid solution was chiefly employed for fixation and microtome sections 6–8  $\mu$  thick were made by the usual paraffin method. For staining, Newton's gentian-violet iodine, safranin light-green, and Heidenhain's iron-alum haematoxylin were used.

## DESCRIPTION

The megaspore mother-cell becomes differentiated shortly before pollination. In the beginning it is only slightly larger than the neighbouring cells, but soon increases in size, becomes three or four times as large as the sterile cells and sharply distinguishes itself from its surroundings (Fig. 1). The cell is very densely packed with starch grains (Fig. 2). According to LAWSON, in this plant there are usually three or four megaspore mother-cells in the same nucellus and since each cell gives rise to four megaspores, twelve or sixteen megaspores are thus formed, but only the one centrally situated is destined to germinate. It is remarkable, however, so far as my observation goes, that only one megaspore mother-cell exists; the cell

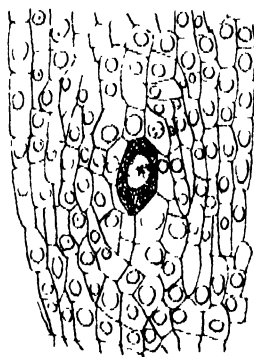


Fig. 1. A longitudinal section of a nucellus showing a single megaspore mother-cell (March 28).  $\times 213$ .



is situated near the base of the nucellus, slightly above the point of the insertion of the integument.

About half a month after the differentiation of the megaspore mother-cell, the reduction division takes place.\* Figures 2 to 6 are the successive

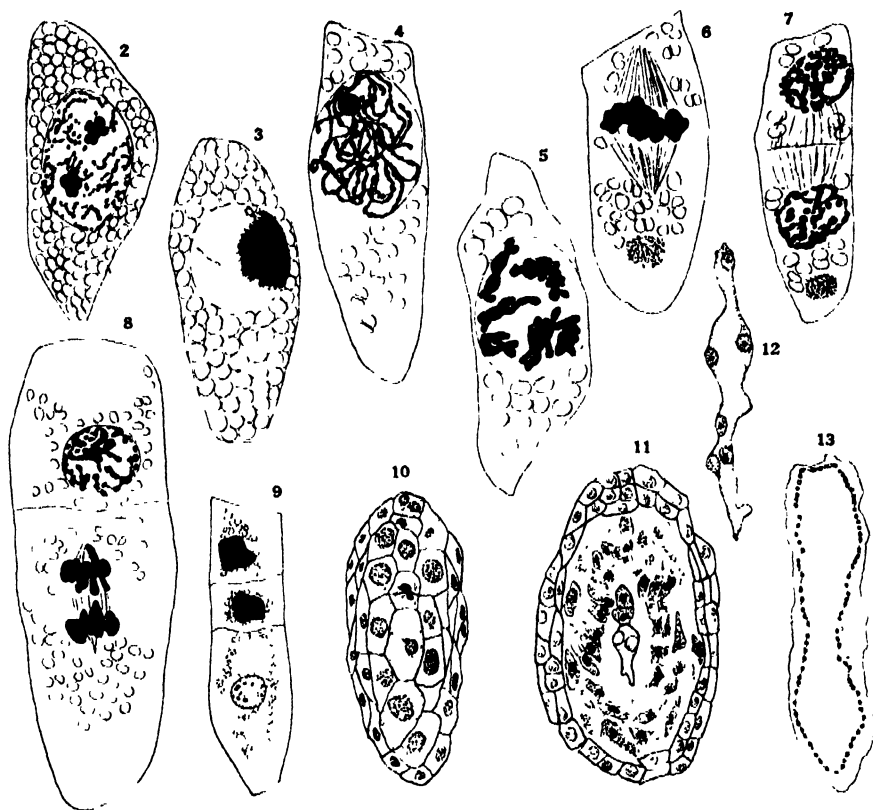


Fig. 2. The megaspore mother-cell preparing for the reduction division. (March 26.)  $\times 910$ . Fig. 3. The same in synapsis. (March 30.)  $\times 580$ . Fig. 4. The same in spireme stage. (April 17.)  $\times 910$ . Fig. 5. The same in diakinesis. (April 20.)  $\times 910$ . Fig. 6. The same showing the spindle of the first division. (April 20.)  $\times 910$ . Fig. 7. The same showing the telophase of the first division. (April 22.)  $\times 910$ . Fig. 8. The same showing the second division in anaphase. (April 20.)  $\times 910$ . Fig. 9. One functional and two abortive megaspores (April 23.)  $\times 580$ . Fig. 10. Tapetal cells surrounding the megaspores. (May 1.)  $\times 213$ . Fig. 11. Binucleate female prothallium with disorganizing tapetal cells. (May 7.)  $\times 213$ . Fig. 12. Young female prothallium in eight nucleate stage. (May 15.)  $\times 213$ . Fig. 13. A longitudinal section of a young female prothallium. (June 15.)  $\times 23$ .

\* LAWSON did not study the details of this division.

stages of the first part of this division. Fig. 3 shows the synapsis, Fig. 4 the spireme and Fig. 5 the diakinesis. In the metaphase of the first division (Fig. 6), a mass of dense cytoplasm is seen in the lower part of the cell. COKER (1) observed a similar structure in *Taxodium* and Miss OTTLEY (2) in *Juniperus*; at present its significance is totally unknown. Large starch grains are observed through all stages of the reduction division.

After the heterotypic division, the megaspore mother-cell is divided into two cells; the upper one is much smaller than the lower (Fig. 8). The lower cell immediately prepares for a second division, while the upper one remains unchanged. Therefore after the second division, only three, not four, cells are formed (Fig. 9).

According to LAWSON, there is no tapetum in *Cryptomeria*. My observation shows, however, that the cells surrounding the megaspore mother-cell contain a large nucleus and small starch grains, and increase in size up to the reduction division (Fig. 10). This distinct zone of large celled tissue disorganizes upon the germination of the megaspore and gives nourishment to the young prothallium (Fig. 11). It may therefore be regarded as a tapetum.

The lowest one of the three cells now begins to enlarge to perform successive free nuclear division, while the other two and the tapetum show all stages of disorganization. The growth of the young prothallium is very slow and it takes about a month to attain to the stage shown in Fig. 13, from the stage in Fig. 11. A higher magnification of the parietal protoplasmic layer is shown in Fig. 14. After a large number of free nuclei have been formed, walls appear among them. The cells thus formed are open toward the central vacuole, the cytoplasm with nucleus being mainly accumulated always on the open side (Fig. 15). A tangential section through the parietal layer in this stage is shown in Fig. 16. The cells in this layer grow then toward the center of the sac, until the sac becomes filled with tissue. Up to this stage LAWSON's descriptions agree very closely with mine. But his statement for the further development of the endosperm is quite peculiar. He says: "The primary prothallial cells soon become multinucleate. The membranes of these primary cells are very delicate and incomplete, and eventually take no part whatever in the formation of permanent cell-walls of the cellular endosperm. The cell walls are formed as a result of a peculiar method of free cell-formation."

My observation concerning the cell-wall formation in the permanent

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tissue of the endosperm has brought light, however, nothing peculiar to this plant. The nuclei which had remained so far at the periphery toward the center of the sac, now move back to middle of the cells as shown in Fig. 18. In this position, mitosis occurs almost simultaneously throughout all the cells of the prothallium. The cell plates are formed between the

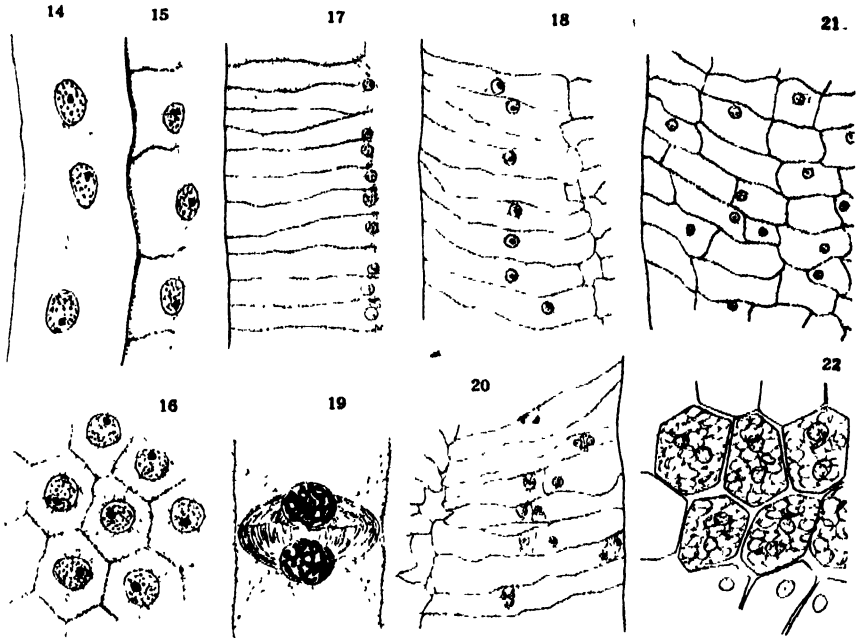


Fig. 14. A longitudinal section of the parietal layer of the female prothallium. (June 17.)  $\times 435$ . Fig. 15. The same showing a later stage: the membranes are formed between the free nuclei. (June 13.)  $\times 36$ . Fig. 16. A tangential section of the same. (June 17.)  $\times 435$ . Fig. 17. Elongated primary prothallial cells with nucleus along the inner exposed surface. (June 17.)  $\times 200$ . Fig. 18. Prothallial cells after the formation of the closing walls; nuclei have moved toward the middle of the cells. (June 19.)  $\times 200$ . Fig. 19. The first division of the prothallial cells. (June 18.)  $\times 405$ . Fig. 20. Prothallial cells each of which are divided into two cells. (June 16.)  $\times 200$ . Fig. 21. The same in a later stage. (June 18.)  $\times 200$ . Fig. 22. The binucleate permanent endosperm cells. (August 14.)  $\times 213$ .

daughter nuclei as usual (Fig. 19). By this division each primary parietal cell is divided into two cells. Divisions of the same kind are repeated several times and rows of cells radiating from the center of the sac are formed. By the time of fertilization the permanent endosperm cells are uninucleate, but afterwards binucleate cells become very common. Whether

# ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES

## III. THE HISTOLOGY OF THE BLOOD OF SOME JAPANESE ASCIDIANS<sup>1)</sup>

By

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(With Plate III and seven text-figures)

(Received May 25, 1936)

During the summers of 1934 and 1935, I made a study of the blood of four species of Japanese solitary ascidians. The work was conducted at the Marine Biological Station of Asamushi. For his valuable suggestion and the kindness of reading the manuscripts, I gratefully acknowledge my indebtedness to Dr. S. HATAI. The material consisted of four species of ascidians; *Cynthia roretzi* v. DRASCHE, *Styela clava* HERDMAN, *Chelyosoma siboga* OKA, and *Corella japonica* var. *asamushi* OKA. The specimens were collected exclusively in the vicinity of the Marine Biological Station and kept in the aquaria.

The blood was withdrawn from the heart through a hypodermic needle after dissection of the test and mantle. The blood corpuscles were studied essentially using the methods of vital and supravital staining with carmine, neutral red, trypan blue and so forth. The influence of some chemical reagents such as acetic acid, caustic soda, hydrogen peroxide, etc. on the blood corpuscles was also observed. The procedure of the vital staining was pursued after the manner described in my foregoing paper (1934). The staining after fixation, using GIEMSA's, MAY-GIEMSA's, PAPPENHEIM's, WRIGHT's stain etc. resulted usually in failure owing to the high concentration of salts in the blood.

### OBSERVATIONS

In the classification of blood cells I followed essentially the example of GEORGE (1926, '30 a and '30 b), and the following types of cells are distinguishable in the fresh blood: 1) green cells, 2) orange cells, 3) brown cells, 4) grayish olive cells, 5) colourless morula cells, 6) hyaline

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<sup>1)</sup> Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 136.

amoeboid cells, 7) granular amoeboid cells, 8) compartmental amoeboid cells and 9) vesicular amoeboid cells. This classification is not absolute, for there are many intermediate types between each kind of blood cells. In the cytoplasm of granular or vesicular amoeboid cells, for example, green or brown bodies are found upon rare occasions. The difference between compartmental amoeboid cells and vesicular amoeboid cells also is not distinct.

The distribution of various blood corpuscles in the blood of four ascidians are given in Table 1.

TABLE 1.

*The distribution of various blood cells in the blood of four ascidians.*

	<i>Cynthia roretzi</i>	<i>Styela clava</i>	<i>Chelyosoma sibeja</i>	<i>Corella jap. var. asamushi</i>
Green cells	+	###	##	+
Orange cells	+	++	—	++
Brown cells	+	++	+	—
Grayish olive cells	—	—	—	+
Colourless morula cells	++	+	—	+
Hyaline amoeboid cells	+	+	+	+
Finely granular amoeboid cells	+	##	—	+
Coarsely granular amoeboid cells	+	++	+	+
Vesicular amoeboid cells	+	+	++	+
Compartmental amoeboid cells	+	—	++	+

1) *Green cells.* These corpuscles are 6–18  $\mu$  in diameter (Plate III, figs. 1–10). In the cytoplasm they contain green granules varying in number from one to twenty or more. The shade of green varies remarkably among individuals and cells even in the same individuum—pale green, greenish yellow or emerald green. The shape of green bodies is usually ovoid or spherical, but occasionally cells are found in which the bodies are rod-, V- or U-shaped. The size of green bodies varies from

0.6 to  $3.5\mu$  in diameter, and is independent of the size of cells. The nucleus is round or oval, and located excentrically owing to the presence of green bodies. The nucleus of certain green cells is covered with green bodies so entirely that it is invisible on the surface. The ratio of the volume of a nucleus to that of cytoplasm is usually low. In the majority of cases, the cytoplasm is clear and homogeneous, but sometimes there is seen the existence of fine or coarse, colourless granules (*Styela*). The large vacuoles are also seen in the cytoplasm of green cells of *Chelyosoma* upon rare occasions. The number and variation of types of green cells are greatest in the blood of *Styela*, and next in *Chelyosoma*. In the blood of *Cynthia* and *Corella*, they are found very infrequently, and the green bodies are small in size and few in number. The reaction of the green bodies to the various reagents is stated under another heading.

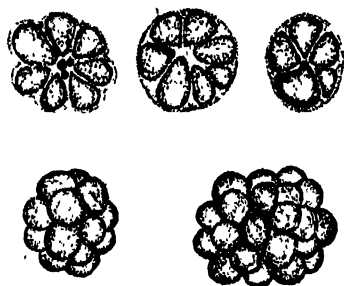
2) *Grayish olive cells*. These cells are found only in the blood of *Corella* and very infrequently. They show a great variety in size. The smaller one is  $3-8\mu$  in diameter, round in shape and the cytoplasm is stained homogeneously with grayish olive pigment. These cells are non-amoeboïd. The larger ones are  $8-16\mu$  in diameter, round or oval in shape, and grayish olive or brownish green coloured granules are found in the cytoplasm scattered or compact. The small, round or oval nucleus, situated in the middle of the cell body is covered occasionally with these granules (Plate III, figs. 11-13).

3) *Orange cells*. The cells are  $6-16\mu$  in diameter (Plate III, figs. 14 and 15). These cells have a cell body of clear cytoplasm in which small, spherical and orange coloured bodies are embedded. The diameter of these bodies measures from one to three  $\mu$ . The orange cells of *Corella* which are round, oval or kidney shaped, have no such granule while the cytoplasm is stained homogeneously with orange pigment. The nucleus is small in size, spherical or oval in shape, and located excentrically. The orange bodies do not show any change in dilute acetic acid, caustic soda and ammonia. The orange cells are very infrequent in the circulating blood, and I failed to detect these cells in the blood of *Chelyosoma*.

4) *Brown cells*. The cells are  $6-13\mu$  in diameter (Plate III, figs. 16-19). These cells consist of cytoplasm in which brown-coloured granular masses of varying size ( $0.5-2\mu$  in diameter) are embedded. The brown masses are round, oval, rod, or polygonal in shape, and sometimes they fuse together into an irregular shaped mass. The round, oval or cocoon-shaped nucleus is located excentrically, and the nuclear volume is usually small. In the cytoplasm, the presence of from one to several vacuoles is

occasionally seen (*Styela*). Fine, colourless granules arranged loosely or compactly are found in the cytoplasm of some brown cells upon rare occasions.

5) *Colourless morula cells*. The cells are 8–18  $\mu$  in diameter (Text-fig. 1). The cells may be divided into two subtypes. The one has a

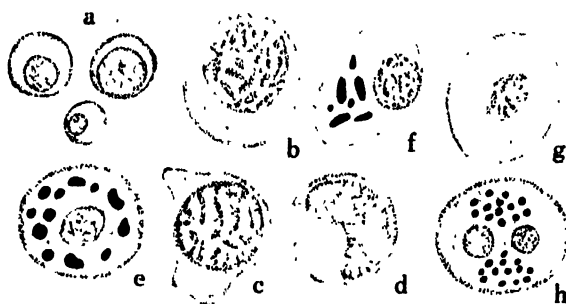


Text-fig. 1. Colourless morula cells from the blood of *Cynthia roretzi*.  $\times 1,000$ .

clear cytoplasm in which from three to several colourless, large granules are arranged in a rosette shape, leaving the cytoplasmic center unoccupied. The granules turn ovoid by mutual pressure, and their narrower ends point to the center of cell. The other in which the cytoplasm is filled up exclusively with round granules about equal in size (approximately two  $\mu$  in diameter) shows a typical morula shape, and the nucleus is quite invisible on account of these granules. There are some transitional

cells between these two types. Colourless morula cells are very frequent in the blood of *Cynthia*.

6) *Hyaline amoeboid cells*. As text-fig. 2 shows, these cells have a perfectly homogeneous cytoplasm. They send out fine pseudopodia, by means of which they move, and show an intensive reaction to the vital



Text-fig. 2. Hyaline amoeboid cells from the blood of *Cynthia roretzi*.  $\times 1,000$ . a, Cells of small lymphocyte-type. b–d, Cells of monocyte-type. e–h, Cells of large lymphocyte-type. The black rods or points show the vital granules of neutral red.

and supravital staining. These cells agglutinate each other *in vitro*. In these cells three subtypes are distinguishable.

i. Cells of small lymphocyte-type, an almost spherical corpuscle about  $3-10\mu$  in diameter. The nucleus is spherical or oval, and some are so large that they occupy the greater part of cell volume while others are so small that their volume does not amount to one tenth of the whole of the cell body.

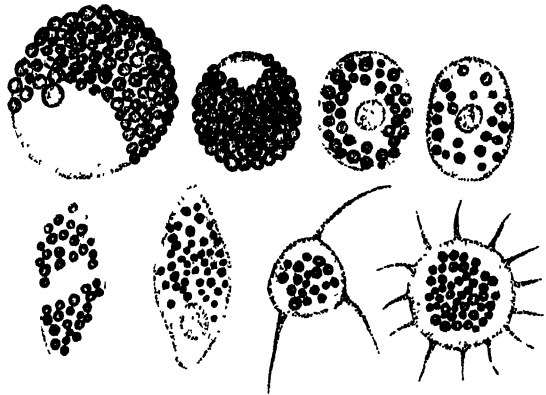
ii. Cells of large lymphocyte-type,  $10-20\mu$  in diameter. These cells have relatively small, oval or spherical nucleus which is located usually in the cytoplasmic center. Binucleated cells are also found occasionally.

iii. Cells of monocyte-type. These cells are similar in every way to the type (ii) save that their nucleus which is situated excentrically is very large and usually kidney or bean-shaped. The nucleolus and coarse granules of chromatin are visible in the nucleus.

Hyaline amoeboid cells which enclose a single or two green cells in their body are found occasionally (Plate III, figs. 9-10). In the blood of *Perophora viridis*, GEORGE (1926) reported also the presence of the large granular amoeboid cell with a green cell or an orange cell, or both, or a cell of signet-ring type enclosed in it.

7) *Granular amoeboid cells*. These are colourless cells having a thin, clear ectoplasm and finely or coarsely granulated endoplasm. The granules of endoplasm are acidophilic, basophilic or amphophilic. There is no evidence of the polymorphism of the nucleus in the present case while the granulocytes of the vertebrates have usually polymorphic lobular nuclei. Granular amoeboid cells are actively amoeboid and phagocytic, and show a positive reaction to the vital and supravital staining. These cells have a tendency to agglutinate into numerous small masses *in vitro*, mixing with the other types of amoeboid cells. Granular amoeboid cells are distinguishable into the following two subtypes.

i. Finely granular amoeboid cells,  $8-16\mu$  in diameter (Text-fig. 3).



Text-fig. 3. Finely granular amoeboid cells from living blood of *Styela clava*.  $\times 1,000$ .



The endoplasm is filled with granules which are nearly uniform in size and somewhat less than one  $\mu$  in diameter. The nucleus is round or oval, and more or less centrally located. Sometimes the nucleus is entirely covered with granules and is hidden from sight. The nuclear volume is rather small. The cells are actively amoeboid, protruding lobular pseudopodia. They take neutral red, trypan blue and so forth *intra vitam*. This kind of cells are very abundant in the blood of *Styela* while I failed to find them in that of *Chelyosoma*.

ii. Coarsely granular amoeboid cells, 8–16  $\mu$  in diameter (Text-fig. 4). The endoplasm is coarsely granular, the ectoplasm is clear and denser. The size of granules shows a remarkable fluctuation: 1.0–3.5  $\mu$  in diameter. The nucleus is located centrally and its volume is not large. These cells are found in the blood of all of the four ascidians, but not so

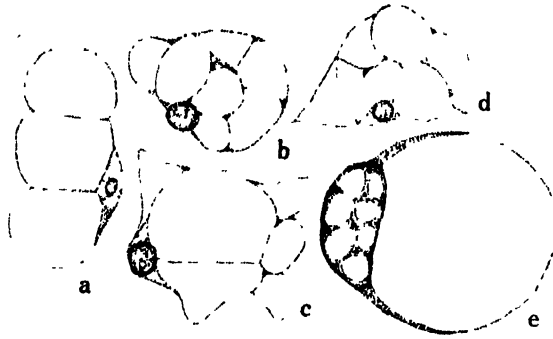


Text-fig. 4. Coarsely granular amoeboid cells from the blood of *Styela clava*.  $\times 1,000$ .

abundant. That there is a difference between fine and coarse granules in respect of their chemical property is very positively shown by means of the oxydase test; namely the latter are intensively positive to SCHULTZE's reaction while the former do not show any change, as it will be described in detail under another heading.

8) *Compartmental amoeboid cells*. The cells are 8–20  $\mu$  in diameter when spherical (Text-fig. 5). In the cell bodies of these, there are a number of vacuoles or compartments separated from one another by partition of the clear, non-granular cytoplasm continuous with the ectoplasm and with the cytoplasm around the nucleus. These vacuolar compartments are round or angular, and the number varies in different cells. These vacuoles are filled with colourless fluid containing very fine granules which show the active Brownian movement. The granules are stained well with methylen blue, neutral red etc., and the fluid of these vacuoles shows also slight colouration with these dyes. GEORGE (1926) reported a very curious behaviour of the dancing granules of *Perophora viridis* when a suitable vital stain is applied; he stated that they agglutinated into masses and rods and continued the quivering movement. I put Japanese

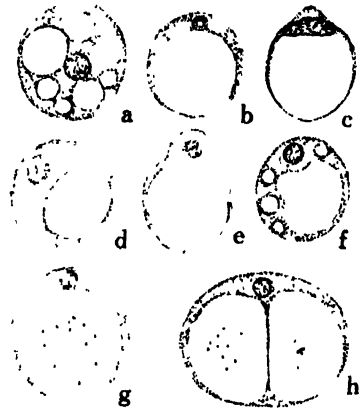
ascidians under repeated observations for this interesting phenomenon, but without success. These cells are slightly amoeboid when they are young,



Text-fig. 5. Compartmental amoeboid cells.  $\times 1,000$ .  
a-d, *Chelyosoma siboja*. e, *Cynthia roretzi*.

while the full grown cells with many vacuoles are inmotile. The compartment cells are frequently present in the blood of *Chelyosoma*, but very rare in that of *Cynthia* and *Corella*, and utterly absent in that of *Styela*

9) *Vesicular amoeboid cells*. The cells are  $6-18 \mu$  in diameter (Text-fig. 6). These cells are very much similar in appearance to the ordinary adipose cell and are found both in the blood and in the test. In the younger cell there are one or several small vacuoles in its clear cytoplasm, and a small and round nucleus is located centrally or excentrically. These vacuoles gradually increase in size with the union of smaller vacuoles with one other, until they form at last a very large single vacuole which occupies almost the whole of the cell body. The cytoplasm is pressed against the cell wall turning into the shape of a signet-ring, and the nucleus is located at the thickened rim of the cytoplasm. Within the vacuoles is fluid which takes methylen blue and neutral red and is stained similar to



Text-fig. 6. Vesicular amoeboid cells.  $\times 1,000$ . a & b, *Styela clava*. c, g & h, *Chelyosoma siboja*. d & e, *Cynthia roretzi*. f, *Corella japonica*.

the fluid in the vacuoles of the compartment cells. In the fluid of some of these cells no granules exist while in the other there are a few dancing granules which strongly reflect the light. These granules have also an affinity for methylen blue and neutral red. Vesicular cells are also amoeboid when they are young, but they become less active, and, at last, immovable with the increase of the volume of vacuoles. Vesicular cells are most abundant in the blood of *Chelyosoma*.

*On some properties of ascidian blood corpuscles*

Under the following five headings some properties of ascidian blood corpuscles are briefly described.

1. *Reaction to the various stains.*

i) Staining after fixation. As I already mentioned above, the staining with GIEMSA's, MAY-GIEMSA's, UNNA-PAPPENHEIM's, WRIGHT's, EHRLICH-BRONDI's stain and so forth ended in failure owing to the high concentration of salts in the blood.

ii) Supravital staining with GIEMSA's stain. The supravital staining with GIEMSA's fluid was made with success by NAKANOIN (1920) on the blood of fishes, and NAGAI (1924) on that of birds. The present author made a trial of this method on the blood of ascidians and obtained satisfactory results. As the boundary between the supravital and post-mortal staining is not distinct, so I considered that it is supravital as long as a nuclear staining does not take place. Four drops of GIEMSA's original solution were added to 2 c.c. of distilled water, and two drops of blood were stained with one drop of the same diluted GIEMSA's solution.

Cells which show an intensive reaction to this staining are chiefly hyaline and granular amoeboid cells, colourless morula cells and vesicular cells. Hyaline and granular amoeboid cells contain granules which stained blue within the first five minutes, then the number of purple or reddish granules gradually increases. After about 30–60 minutes I observed a cell which contains red granules only, showing the presence of eosinophilic granulocytes. I made also a supravital staining with 0.01 per cent solution of Azur II, and the presence of azurophilic granules was ascertained. These granules supravitaly stained were usually round, but the presence of ellipsoid or rod-shaped granules was also detected occasionally.

iii) Vital staining. KRYONO (1928) described that the blood corpuscles of *Cynthia karasboja* show a positive reaction to the vital staining with lithium carmine. I tried this method repeatedly, but with failure. Trypan

blue is ingested by hyaline and granular amoeboid cells, but not intensive. Neutral red shows a great affinity to the blood corpuscles except the orange cells and morula cells. The vital granules of neutral red are usually round in shape, fresh red in colour, and show a remarkable fluctuation in size. The fluid of vesicular amoeboid cells are stained purplish red with neutral red, which shows the acidity of the content.

### 2. *Oxydase reaction.*

Green, brown, morula, hyaline, granular, and vesicular cells are positive to SCHULTZE's oxydase reaction. In the morula cells and coarsely granular amoeboid cells, the preexisting granules turn blue immediately, showing the presence of oxydase in the granules themselves, and the oxydase granules which are newly produced are not seen entirely (Plate I, figs. 22-24). In the green cells, the green granules turn greenish blue at first, then, after long exposure to the reagent, these granules begin to dissolve into the water, perhaps owing to the action of the caustic soda contained in the solution. In addition to these changes, the fresh appearance of oxydase granules, which are numerous, fine, and round, is observed in the cytoplasm. The preexisting granules of brown cells and those of finely granular amoeboid cells show no sign of indophenol synthesis, while the intergranular cytoplasm show it very positively (Plate III, fig. 25). In the cytoplasm of hyaline amoeboid cells and also in that of vesicular cells the oxydase granules are discovered, but more intensively visible in the former than in the latter.

### 3. *Properties of granules with respect to chemical reagents.*

The granules of ascidian blood corpuscles drew the attention of many investigators such as HARLESS (1847), KRUCKENBERG (1880), CUÉNOT (1891), HENZE (1911, '12), HECHT (1918), GEORGE (1926, '30 a, '30 b) and so forth. I made also some observations on the chemical property of these granules.

i) Distilled water. There is no immediate effect on the granules, they remain intact at the end of 30 minutes.

ii) Alcohol and ether. HARLESS (1847) reported that some colourless granular amoeboid cells of *Phallusia* show a deep blue colouration in alcohol or ether, and which was confirmed by KRUCKENBERG (1880). Under the present investigation, however, any change of colour due to the action of alcohol and ether was not recognized. Alcohol (70 per cent or absolute) does not destroy the granules, which seem, however, to become coagulated gradually.

iii) Iodine. Diluted solution of I-IK turns the granules of colourless morula cells dark green.

iv) Sudan III and Scharlach R. GEORGE (1926) described that some granules of granular amoeboid cells and dancing granules found in the vacuoles of the compartmental amoeboid cells of *Perophora* stain with Sudan III and Scharlach R. I also observed the same phenomenon in the granular amoeboid cells of *Styela*.

v) Osmic acid. The granules mentioned just above are slightly blackened by osmic acid.

vi) MILLON's reagent. Some granules of colourless amoeboid cells undergo a brick red colouration while others do not.

vii) Aceton and hydrogen peroxide. The green granules of green cells change their shade and turn gradually orange or brownish colour. This reaction hints at the existence of a relation among green, brown, and orange cells.

viii) Ammonia. Ammonia turns green granules yellowish. The granules of colourless morula cells show dark greenish colouration with the application of ammonia. They are partly dissolved with ammonia and fuse one into another forming granules.

ix) Caustic soda. Dilute solution of caustic soda dissolves the green body slowly, leaving the vacuolated cell with an excentrically placed nucleus.

From the last three reactions it is possible to conclude that the ascidian blood pigments under the present investigation are also the vanadium compounds, as it was already reported by HENZE (1911, '12).

#### 4. *Amoeboid movement.*

The amoeboid movement of ascidian blood corpuscles has been reported by many authors; CUÉNOT (1891), HECHT (1918), FULTON (1920), GEORGE (1926, '30) etc. GEORGE described five types of amoeboid cells—green, orange, granular, vesicular, and signet-ring type cell, while FULTON maintained that the last type of cell is not amoeboid. The observation of the present author is in accord with that of GEORGE. The young type of compartmental cells and vesicular cells are distinctly amoeboid, though their full grown types are immovable.

In the hypotonic sea water the pseudopodia of blood corpuscles gradually disappear and the cell bodies expand till their shape becomes almost spherical. The granules of the expanded amoeboid cells accumulate at the center of cell, and the cell-periphery becomes transparent. At the immediately later period of this stage the tongue-like processes are usually produced, till at last the cell membrane, which is very clearly distinguishable from the cytoplasm, gives way and the granules escape.

In the hypertonic solution, the amoeboid cells become to remain still,

taking the spherical or oval shape. In a solution which has an osmotic pressure twice that of sea water, the amoeboid cells gradually change their shape, and sometimes thread-like pseudopodia are observed. Afterwards, considerable shrinkage occurs, though cytolysis does not take place immediately.

A most active amoeboid movement was observed in the finely granular amoeboid cells of *Styela clava* (Text-fig. 7). Thin lamella of clear cytoplasm flows outwards from all parts of the cell along the margin of the



Text-fig. 7. Amoeboid movement and phagocytosis of the finely granular amoeboid cells from the blood of *Styela clava*.  $\times 800$

cell body, then the endoplasm which contains many granule streams continuously into the extruded ectoplasm. The extrusion of pseudopodium is usually in one direction, but pseudopodia which are extruded in two or three directions, forming V-, W-, or Y-shape, are seen not infrequently. As a type of amoeboid movement, a surprising elongation of cell-body is recognized. One end of the cell is fixed on the surface of the slide glass, and the free end is sent forth as a pseudopodium. The cell increases greatly in length by the elongation of this pseudopodium, changing its form from the initial leech-like shape to that of a fine thread by and by. In an extreme case the length of a cell reaches  $180\ \mu$  or more while its breadth is only 3 or  $4\ \mu$ . Both ends and two or three regions in the middle of the cell remain swollen, and the granules are seen only in these regions.

Finely granular amoeboid cells are actively phagocytic too. Under the microscopic observation, the amoeboid cell elongates its body till it

reaches the object such as carbon particles which were previously introduced to the blood. Then the pseudopodia are extruded in the shape of a V and the carbon particles is surrounded with these, till at last the particle is enclosed entirely in the cell body; pseudopodia disappear and the cell recovers its original shape.

The amoeboid movement is sensitive to the changes of the environmental factors. Therefore, an application of acids or alkali, change in osmotic pressure etc. cause the interruption of the movement.

##### 5. Agglutination.

The blood of ascidians does not coagulate, but the amoeboid cells constantly gather together in irregular clumps, as is seen in the blood of many invertebrates. GEDDES (1880), THÉEL (1920, '21) and others believed that agglutinated cells are completely fused together into a mass comparable to the plasmodia of *Myxomycetes*. TAKATSUKI (1934) did not find such a real plasmodium formation of the blood corpuscles of the oyster, but he admitted that the boundaries of the cells which are located at the center seem gradually to disappear and become more or less structureless, resembling a mass of protoplasm. The present author (1934 a, '34 b) also reported the similar result on this point in the previous paper which treated of the blood of a holothurid and an earthworm. In the present investigation it was also testified by the blood of ascidians. The cells which composed the agglutinated mass are chiefly hyaline and colourless granular amoeboid cells.

#### COMPARISON AND DISCUSSION

Our knowledge of the ascidian blood is due chiefly to the studies of CUÉNOT, KOLLMANN, HENZE, FULTON, HECHT, SIMPKINS, GEORGE and to the observations of a number of other investigators. The classification of ascidian blood corpuscles greatly varies with different authors. The excellent and detailed classifications were made by two authors: FULTON and GEORGE. FULTON (1920), working with the ascidian of Bermuda (*Ascidia atra*), divided the blood corpuscles into ten kinds: 1) Green cells. 2) Orange cells. 3) Blue cells. 4) A-1 Homogeneous amoeboid cells. 5) A-2 Granular amoeboid cells. 6) A-3 Granular cells that swim. 7) Q-1 Spherical cells with a single large vacuole. 8) Q-2, similar to Q-1. 9) Q-3 A bilobed cell with hyaline cytoplasm. 10) Q-4 A tripartid cell. He mentions in addition a cell with brown granules, rare in occurrence. GEORGE (1926, '30 a, '30 b) observed seven species of ascidians native in Beaufort

and Bermuda. He distinguished nine types of blood corpuscles: 1) Green cells, 2) Orange cells, 3) Brown cells, 4) Blue cells, 5) Colourless morula cells, 6) Finely granular amoeboid cells, 7) Coarsely granular amoeboid cells, 8) Signet-ring type of cell, and 9) Compartmental amoeboid cells. Besides these he also mentioned brownish cells and large greenish blue cells of the blood of *Symplegma viride*. As I already mentioned above, I followed this classification in general. I wish here to compare my result with that of GEORGE, in reference to the report of FULTON.

It is apparent from FULTON's description and figures that green cells of *Ascidia atra* are essentially similar to the green cell of various ascidians which are described and figured by GEORGE. The green granules of these assume always the morula-like arrangement, leaving a clear cytoplasmic center intact. In the present investigation, however, such an arrangement was discovered rather rarely, and the green bodies usually grouped in a mass at the center of the cell-body. FULTON stated that, so far as he was able to ascertain, the green cells were non-nucleated and non-amoeboid. GEORGE's observations brought out the reverse results on these points. In the present study the green cells were always nucleated, and the amoeboid movement was very common, sending forth lobular pseudopodia.

The presence of brown cells in the blood of *Tunicata* was described for the first time by FULTON in his remarks upon *Ascidia atra*. GEORGE found the brown cells in the blood of *Clavellina oblongata*, and the brownish cells in that of *Symplegma viride*. He considered that these types of cells might originate from compartmental cells, the same as mulberry coloured cells and greenish blue cells. KIYONO reported also the presence of brown cells in *Cynthia karasboja*. In my case the brown cells were met with rather frequently in the blood of *Styela clava* while GEORGE did not find them in *Styela plicata*. In the blood of *Cynthia roretzi* and *Chelyosoma siboya*, the brown cells were discovered but seldom while they could not be detected in the blood of *Corella japonica* var. *asamushi*.

The presence of blue cells in the blood of *Ascidia atra* was reported by HECHT (1918) and FULTON. GEORGE also described greenish blue cells of *Symplegma viride*. In the present investigation, however, this type of cell was not found in any specimen.

The type of grayish olive- or grayish purple coloured cells which I found in the blood of *Corella japonica* var. *asamushi* is not described in any previous papers, so far as I am aware. Whether this type of cell is specific or not to this species is yet to be solved. GEORGE emphasized



in his article that the green granules show a good deal of variation in the shade of green against chemical and other factors. That the vanadium compounds show various colouration is also evident from chemical analyses. It is possible, therefore, that the tint of grayish olive or brownish green of the aforementioned corpuscles is derived from the green cells under some special conditions. The green cells of *Corella* are relatively small ( $5-8\mu$ ) and few in number (1-3). Grayish olive cells, to the contrary, large in size ( $8-16\mu$ ), and coloured granules are so abundant that they compactly fill up the cell-body. I infer from these facts that the grayish olive cells might stand for an older and full grown stage of green cells. But I failed to detect the presence of successive transitional stages between green cells and grayish olive cells.

FULTON described the perfectly homogeneous amoeboid cells, called 'type A-1', of the blood of *Ascidia atra*. GEORGE stated about this type of cell as follows; "I believe that these cells are probably the same as my amoeboid compartment cells, a type which he did not describe and which appear homogeneous unless closely examined". I carried on careful observations on the hyaline amoeboid cells, keeping in mind GEORGE's description. I found, however, the simultaneous presence of both types of cells in the blood of *Cynthia*, *Chelyosoma* and *Corella*, and hyaline amoeboid cells alone in that of *Styela*. GEORGE stated also that when a weak neutral red in sea water is added to the edge of the cover glass, the hyaline cells of *Phallusia nigra* (*Ascidia atra*) after about half an hour begin to show evidence of being reticulated and of containing angular vacuoles. I repeated this examination on the hyaline cells, but such changes did not take place. Furthermore, the vital staining of hyaline cells with this dye is remarkably positive while that of compartmental cells almost negative. I consider, therefore, that ascidian blood contains the hyaline leucocytes besides the compartmental cells.

A type of cells which bear a close resemblance to the compartmental cells of ascidians was found by THÉEL (1920-'21) in the blood of *Echino-derma*: *Mesothuria intestinalis*, *Psolus phantapus*, *Asterias rubens* and *Parechinus miliaris*, and he named it "bladder-amoebocyte". I also noticed the compartmental cells in the blood of *Molpadia roretzii*, *Temno-pleurus hardwickii*, *Urechis uncinatus* and others. Therefore, this type of cell is rather widely distributed in the blood of invertebrates.

## SUMMARY

1) There are nine kinds of corpuscles in the blood of four Japanese ascidians: green-, orange-, brown-, morula-, grayish olive-, hyaline-, compartmental-, granule- and vesicle-cells.

2) Green granules are dissolved by the action of acids or alkali, and their shade is faded by adding hydrogen peroxide, acetone or ammonia.

3) Hyaline and granular amoebocytes are phagocytic, and show positive reaction to the vital staining with acidic or basic stains.

4) Green cells, brown cells, hyaline amoeboid cells, granular amoeboid cells, morula cells, and vesicular cells are positive to the SCHULTZE's oxydase reaction.

5) The amoeboid cells tend to agglutinate into a mass *in vitro*, but the real plasmodium formation is not visible. There is no trace of coagulation of plasma and fibrin production.

6) Amoeboid movement and phagocytosis are found most intensive in the finely granular amoeboid cells. The normal form of pseudopodia is always lobular or petalloid, and thread or needle-shaped pseudopodia are seen under unfavourable conditions.

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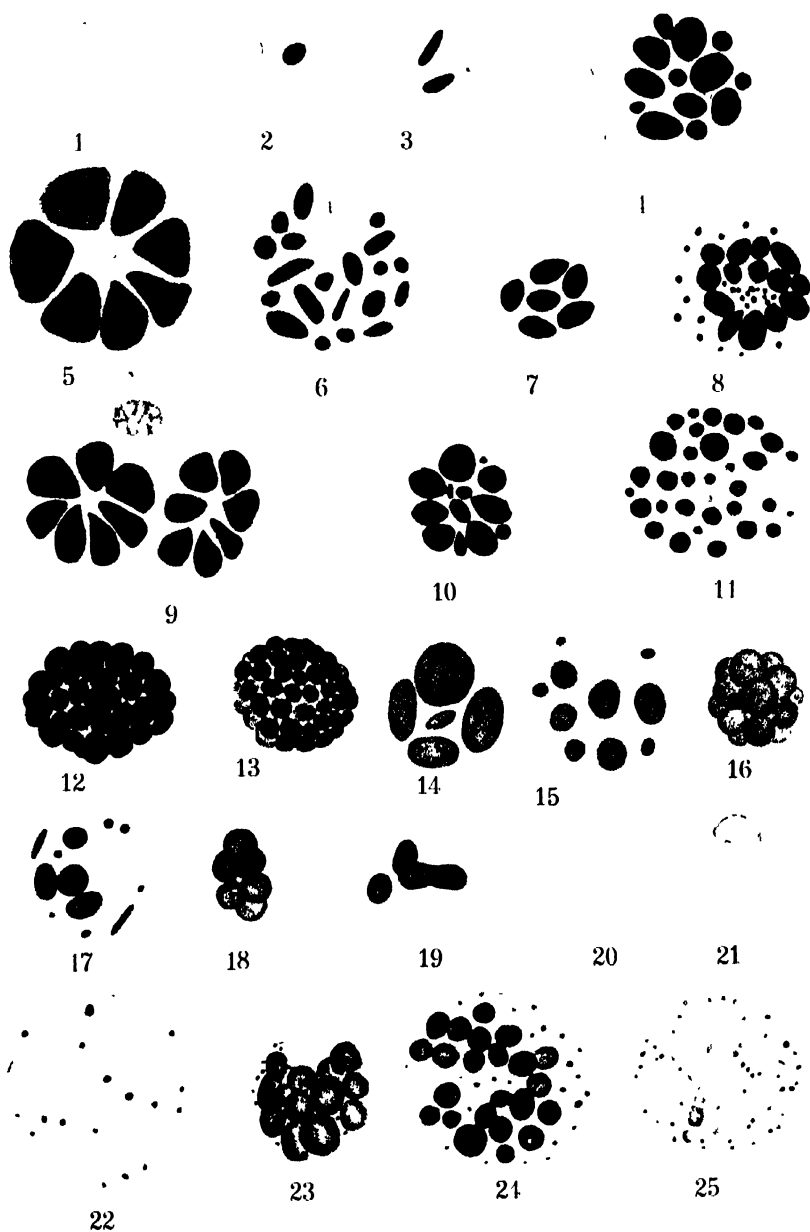
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### EXPLANATION OF PLATE III

All figures are camera-lucida drawing at a magnification of 2,000 diameter.

- 1- 7 Various types of green cells from a cover-glass preparation of living blood unstained (1, *Chelysoma siboga*; 2 and 3, *Corella japonica*; 4-7, *Styela clava*).
- 8 Indophenol synthesis in the cytoplasm and green bodies of a green cell from the blood of *Styela clava*.
- 9-10 Hyaline amoeboid cells with a single or two green cells. From the blood of *Styela clava*.
- 11-13 Grayish olive cells from the unstained living blood of *Corella japonica*.
- 14-15 Orange cells from the unstained living blood of *Cynthia rosetti*.
- 16-19 Brown cells from the unstained living blood of *Styela clava*.
- 20-21 Vesicular cells from the living blood of *Corella japonica*. Fluid of vacuoles is slightly stained with neutral red *intra vitam*.
- 22-25 Indophenol synthesis of colourless morula cell (22), coarsely granular (23 & 24), and finely granular cells from the blood of *Styela clava*.



Author del.

T. OHUYE: Coelomic Corpuscles of Invertebrates, III.



# ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATE

## IV. ON THE COELOMIC CORPUSCLES OF A HOLOTHURID, *MOLPADIA RORETZII* (V. MARENZELLER) WITH REFERENCE TO THOSE OF *CAUDINA CHILENSIS* (J. MÜLLER)<sup>1)</sup>

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(With twelve figures)

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The present paper is a histological study of the coelomic corpuscles of a holothurid, *Molpadia roretzii*. Histology of the coelomic corpuscles of *Caudina chilensis* was reported by KAWAMOTO (1927). The present author (1934) also has published some informations about the reaction of the leucocytes of *Caudia* to vital dyes. The present specimen, *Molpadia roretzii*, belongs to the family *Molpadiidae* the same as *Caudina*. It is natural, therefore, that the coelomic corpuscles of two animals bear a close resemblance to each other. Here I wish to describe the characteristics of the coelomic corpuscles of *Molpadia* in comparison with those of *Caudina*.

The work was undertaken at the suggestion of Assist. Prof. S. KOKUBO, to whom I would express my indebtedness. I am also deeply grateful to Dr. S. HATAI for reading and criticizing the manuscript in spite of his precious time.

The specimens were collected in the vicinity of the Asamushi Marine Biological Station and kept in an aquarium. The coelomic corpuscles were examined by means of supravital staining chiefly, but the methods of the vital staining and staining after fixation were also employed.

### OBSERVATION

From the histological point of view eight kinds of corpuscles are distinguishable: 1) red corpuscles, 2) hyaline amoeboid corpuscles, 3) colourless granular amoeboid corpuscles, 4) brown granular amoeboid corpuscles, 5) vesicular amoeboid corpuscles, 6) crystal corpuscles, 7) spindle corpus-

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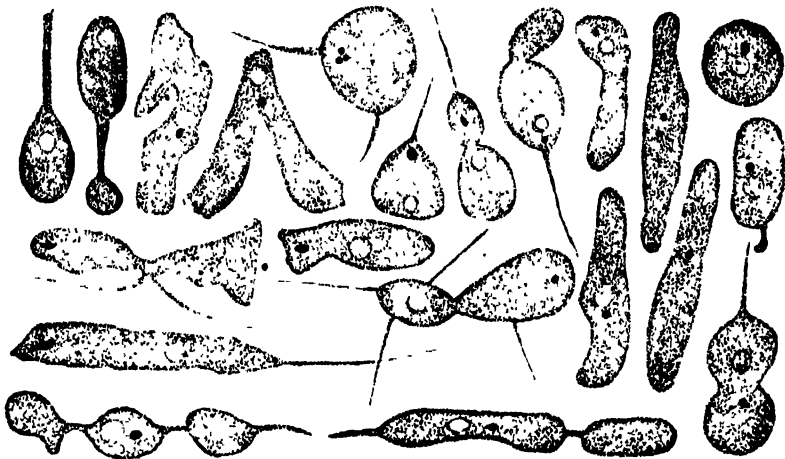
<sup>1)</sup>Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 137.

cles, 8) minute corpuscles and 9) fusiform corpuscles.

1) *Red Corpuscles*.

(a) General properties. When viewed one by one the corpuscles do not appear red, but merely of a pale yellow tinge; a distinct red colour which resembles those of vertebrates is seen when a number of them are brought together. The corpuscles freshly withdrawn are discoidal or oval, flattened and more or less biconvex. Their size differs considerably even in the same individual, the largest measuring  $32\ \mu$  in longer diameter and the smallest  $10\ \mu$ , and there is every intermediate gradation in size between these extremes.

As is seen in *Caudina*, the corpuscles change their forms gradually when removed from the animal body. Under the microscopic observation, the cell seems to swell a little, first, then elongates itself gradually, and takes the shape of a pear or that of I-, or V-letter (Text-fig. 1). Its



Text-fig. 1. Various forms of the red corpuscles of *Molpadia* taken from living specimens.  $\times 1,200$ .

contour becomes erose and the shade of colour seems to fade slightly. Subsequently it assumes an irregular shape by sending forth one or several spine- or bristle-shaped processes. These processes protrude usually from the poles, but they are seen occasionally in the middle part of the cell body.

No branching of spine is found in any specimen. The length of a process varies from  $8$  to  $20\ \mu$ , and the total length of the cell, including the process, reaches  $70\ \mu$  or more in the extreme case. Red copuscles have

the tendency to form cytoplasmic processes more intensely in the isotonic sodium chloride solution than in the isotonic sea water. In the hypotonic solution such as N/4 NaCl-solution or diluted sea water (one forth), the red cells assume always a spherical shape without any protuberance.

In the blood of *Thyone fusus*, THÉEL (1920) observed the roundish protuberances of red corpuscles, and these protuberances appear to separate themselves from the mother cell resulting in the formation of free and hyaline particles. Such phenomenon, however, was not observed in the present case.

That the upgrowth of the pseudopodia-like process is due to the changes taking place under an abnormal condition is clear from the fact that the process is totally absent when the red corpuscles are withdrawn from the blood vessel and fixed very rapidly using BOUIN's fluid, I-IK solution &c.

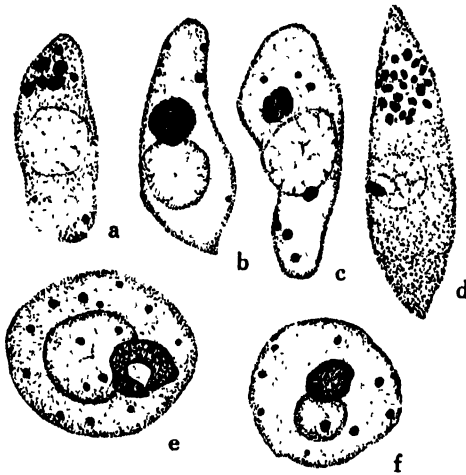
The cytoplasm of corpuscles is almost homogeneous, and lodges one or several refractive granules which are  $1-2.5\mu$  in diameter, yellowish brown in colour, and usually situated in the vicinity of the nucleus or a pole of cell, opposite the nucleus. The roundish nucleus is obscure in the cells freshly withdrawn, but it becomes clearer if one keeps the corpuscles under observation for a while. The nucleus is  $3-4\mu$  in diameter and one or two nucleolei are found in it.

After GIEMSA's stain on dry-fixed smears the nucleus becomes very distinct. Refractive granules either remain unstained or appear brownish black. With the osmic acid they seem to be somewhat blackened. The cytoplasm of the corpuscles stains intensely with eosin except for a narrow peripheral zone. A similar unstained surface zone was described and figured by KAWAMOTO (1927) of the corpuscles of *Caudina chilensis* and by DAWSON (1932) of those of *Thyone briareus*. The red corpuscles are not stained with trypan blue, carmine and so forth *intra vitam*, but the refractive granules become deep reddish brown or brownish blue by the vital staining with neutral red or brilliant cresyl blue, as was seen in the case of *Caudina*, and reported by DAWSON in the case of *Thyone*.

It is very interesting that the red cells, especially refractive brown granules, are positive to SCHULTZE's oxydase reaction (Text-fig. 2). The refractive brown granule is first stained diffusely green or greenish blue, then the shade gradually becomes deeper, and turns at last to blackish blue. After about half an hour or an hour, the refractive granule is divided into small particles, from three to twenty or more. In the cytoplasm, small and round oxydase granules are also visible. The red cell



of *Caudina* shows also a similar behaviour to the oxydase reaction. I suppose, therefore, the refractive brown granules of red cells to be a center of intracellular metabolism. Peroxydase reaction was negative in both *Molpadia* and *Caudina*.



Text-fig. 2. Oxydase reaction of the red corpuscles.  $\times 1,800$ . a-d, *Molpadia*. e-f, *Caudina*.

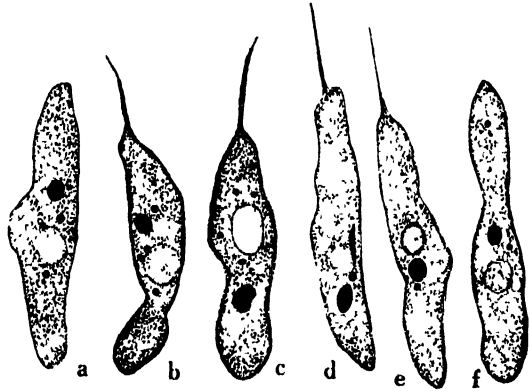
(b) Cytoplasmic fine structures. As for the cytoplasmic components of erythrocytes of the vertebrates, especially amphibians, they were studied by many authors (AVEL 1924, JORDAN 1925, CHLOPIN 1927, DAWSON 1928, '29, '33, GODA 1929, KURASHIGE 1930, TAKAGI 1931, '32, '36, SHIBATA 1932, TOMITA and others 1934, YASUZUMI and others 1934, KAWABE 1934, and so forth) by means of the vital and supravital staining, as well as the silver impregnation, and granular, fibrillar or reticular substances

were demonstrated. In contrast with the abundant researches in the field of vertebrates, the deficiency of attentions paid concerning the coloured corpuscles of invertebrates is remarkable, and only two authors (ROMIEU 1923 and DAWSON 1932) are citable so far as I am aware. ROMIEU found that in *Notomatus* vital staining with the high concentrations of dye caused the appearance of a coarse-meshed network whose branches tend to radiate from the nucleus to the periphery of the cell. He also obtained a somewhat similar pattern in the corpuscles of *Glycera tessellata* after hemolysis and suggested that it might correspond to the reticulation substance of the vertebrate erythrocyte. DAWSON, working with *Thyone briareus*, reported that the mitochondria of red cells are readily demonstrated with Janus green B, and they appear granular at first, but as the preparation ages and Brownian activity becomes more marked they are seen to be short rods orientated at right angles to the flattened surfaces of the cells. He also described and figured a definite filamentous structure in the red cell of *Phascolosoma* and *Glycera* after treated with high concentrations of brilliant cresyl blue. In the red cell of *Arca*, DAWSON found also a variable number of granules or short rods which are stained with Janus

green B, brilliant cresyl blue and neutral red.

In the present investigation, SABIN's method was used for the reaction of red cells to the neutral red. The preparations were sealed by bathing the cover-slips with vaselin, and observations were made on supravital preparations continuously. The same dilution of dye (1 : 1,000 in absolute alcohol) was used for all the blood samples.

In the cytoplasm of *Molpadia* erythrocytes, two to four neutral red bodies appeared bipolarly, showing Brownian movement, within about 20 minutes (Text-fig. 3). It seems to be obvious that these are of a similar substance to the granules which were found in the frog erythrocyte by JORDAN (1925) and named 'segregation apparatus'. DAWSON (1928, '29, '30, '32), working with many species of fish and amphibia, called them 'primary granules'. YASUZUMI and his co-workers (1931) applied the term B-granula



Text-fig. 3. The 'segregation apparatus' of red corpuscles.  $\times 1,600$ . a-c, *Molpadia*. d-f, *Caudina*

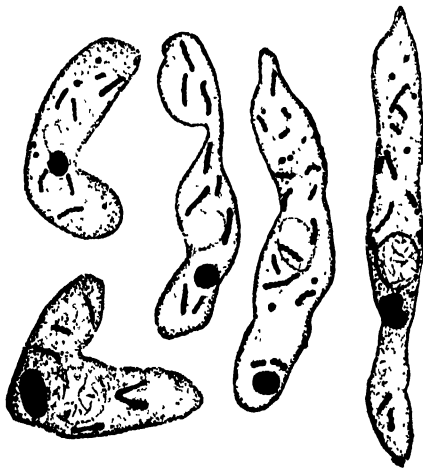
(B means Brownian movement) to those of *Diemictylus pyrrhogaster*. In the red cell of *Caudina*, the granules of the segregation apparatus appear fewer in number than that of *Molpadia*, and usually single or two in unipolar, but occasionally three or more granules in bipolar clusters are visible. DAWSON (1929) determined the amount and distribution of the granules of 'segregation apparatus' in several species of urodeles, and described that bipolar patterns are characteristic of the non-metamorphosed or incompletely metamorphosed forms, while the unipolar pattern is more often found in the erythrocytes of the fully metamorphosed animals, although there are some exceptions to such generalizations. He stated also in another report (1932), that the history of the primary (preexisting) and secondary (induced) granules is less regular and consequently less useful for measuring the relative differentiation attained by the cells of different classes of vertebrates; however, within a given class of vertebrates it is concluded that the presence of a large number of primary granules or the rapid induction of new granules in mature cells may be regarded

as a supplementary evidence of the less degree of differentiation. If the application of this principle to the invertebrates is possible, it is also concluded that the red cell of *Caudina* is in a more differentiated stage than that of *Molpadia*.

Prolonged exposure of red cells to the dye causes the appearance of numerous fine granules. These are called the secondary granules by DAWSON, F-granules (F means fine) by YASUZUMI and others. No difference in the behaviour of secondary granules between *Molpadia* and *Caudina* is discernible.

After about 70 minutes of exposure to the dye, the reticular patterns appear. In the young type of the cell, the cytoplasm is filled with these patterns of reticulation and no granule is visible.

By means of brilliant cresyl blue, the staining of these three kinds of cytoplasmic structures is also successful. The induction of second granules is, however, more quick, and the induced granules are coarser than in the case of neutral red. Janus green B brings out a variable number of small, irregularly scattered granules or short rods which are interpreted as mitochondria.



Text-fig. 1. Silver impregnation of red corpuscles of *Molpadia*.  $\times 1,800$ .

As to the technique of silver impregnation of red cells, I followed the description of SHIBATA (1932) who obtained excellent results, using ALTMANN's solution for fixation and gelatine-silver-nitrate mixture of FUJITA (1929), in the impregnation of amphibian erythrocytes. FUJITA's method was modified recently by TOMITA, FUJITA and IZUMI (1934). Of the result obtained by adopting this modification will be reported on another occasion.

In the cytoplasm of normal red cells, the argentophilic substances appear in the form of numerous and round granules which arrange themselves in straight or spiral threads closely similar to beads in appearance (Text-fig. 4). When observed under lower magnification they may be seen as mere simple and fine threads. The brown refractive granule also blackens slightly.

Red cells which were previously immersed in hypotonic sea water for about an hour or two are also positive to the silver impregnation. In sea water which is diluted with the equal volume of distilled water, the silver images of red cells appear also as coarse and not numerous granules, but they are scattered irregularly in the cytoplasm and do not form the shape of beads. In the case of immersion into a more dilute solution such as sea water 1:distilled water 3, very fine and numerous granules or rods, filling the cytoplasm densely appear. SHIBATA (1932) reported a similar result in the case of *Diemictylus pyrrhogaster*; namely the red blood corpuscles which are immersed in a 0.6 per cent solution of sodium chloride showed very fine and numerous granules instead of spiral or looped threads. The red cell of *Caudina* shows also a similar result in silver impregnation to that of *Molpadia*.

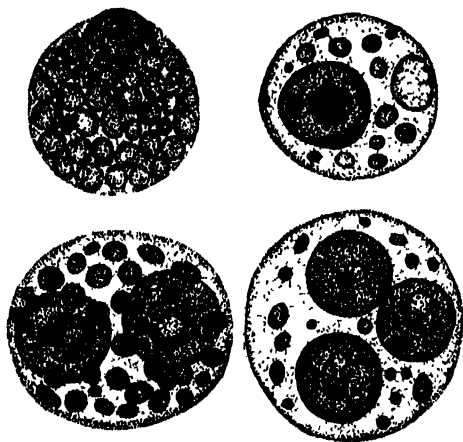
It is obvious from the description just given that the coloured corpuscles of *Molpadia* and *Caudina* bear a close resemblance to those of the vertebrates, in the point of supravital staining with neutral red, brilliant cresyl blue and Janus green B, as well as of silver impregnation.

(c) Erythrocytrophagocytosis. KAWAMOTO (1927) stated that *Caudina* cannot live in the aquarium for more than a week or two even in a favourable temperature, and he supposed that there is some relation between the numbers of corpuscles during this period and the death of *Caudina*.

The present author observed some morphological changes of red cells in the perivisceral cavity of several individuals of *Caudina* which have been kept in an aquarium for about ten days. The striking changes in blood were as follows. In the first place, the entire red cells become round or oval in shape, while they take an elongated spindle shape in the animals which are freshly captured. Secondly, the increase in number of fusiform corpuscles are remarkable. In an extreme case the ratio of fusiform corpuscles to red cells was 20:94. The size of the fusiform corpuscles of freshly captured animals, as KAWAMOTO reported, measure 10–30  $\mu$  in length and 3–8  $\mu$  in width. In the perivisceral cavity of animals captured long since, however, there are found frequently giant types such as measure 120  $\mu$  or more in length and 17  $\mu$  in width. Thirdly the erythrocytrophagocytosis of the macrophages occurs very intensely (Text-fig. 5). In the macrophage, usually single or two, occasionally three or more, and in the extreme cases nine nuclei are found. The cytoplasm is hyaline and clear, but rarely, a remarkable number of colourless or brown granules are visible. The macrophage ingests usually one to three

red cells in its cytoplasm. Surrounding these red cells small and round granules which are stainable vitally and supravitaly with neutral red, brilliant cresyl blue, Janus green B and so forth appear gradually. The contour of the

ingested red cell become irregular and uncertain, but the brown refractive body of it remains for a long period with no remarkable change. The erythrocytrophagocytosis is very common in the course of anemia of the vertebrates. DAWSON (1933) reported also a definite increase of the monocytes in the blood of *Necturus* which is immersed in a dilute solution of lead acetate, and the erythrocytes injured through lead poisoning were ingested by



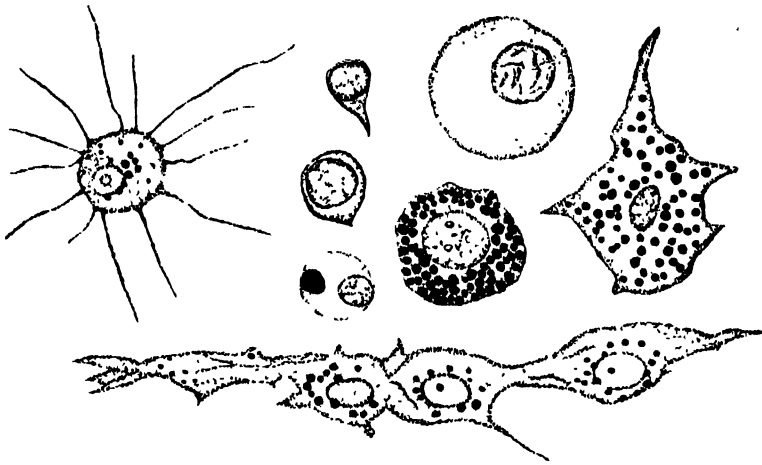
Text-fig. 5. Erythrocytrophagocytosis of macrophages from the living blood of *Caudina*. Vital granules of neutral red are seen in the cytoplasm of macrophages.  $\times 1,200$ .

these monocytes. The blood picture of *Caudina* during the second seven days of captivity is very similar to that of vertebrates in an anemic condition due to the action of blood poisons. Is not the damage of red cells, I suppose, a cause of death of *Caudina* in the aquarium? As already KAWAMOTO described, the number of red cells in the perivisceral cavity and water canal of *Caudina* show a tendency of decrease within the first seven days of captivity. In order to cause intensive anemia, I introduced 0.5 c.c. of one per cent solution of phenyl-hydrazine two or three times intraperitoneally with an interval of 24 hours, and blood was taken and examined five days after the last injection. The animals which received such injections became pale or brownish, shrink vigorously, and came out from the sand in which they usually embed their own bodies. Severe anemia occurred in these animals, and the erythrocytrophagocytosis and increase of fusiform corpuscles were also remarkable. The compensatory erythropoiesis, however, was not determined, because these animals died on the height of severe anemia. The changes of the blood of *Molpadia* were similar to those of *Caudina*, but *Molpadia* is not suitable for the experiment on account of its quicker emaciation and collapse. It seems to me that there still remain to be solved many interesting pro-

blems concerning the changes of blood of *Caudina* in the aquarium and the hemopoiesis in the course of recovery from the anemia caused by unfavorable feeding conditions or poisoning. Further investigations are necessary on these subjects.

## 2) *Hyaline Amoeboid Corpuscles.*

This is the "leucocyte" in the term of KINDRED and "hyaline plasmamoebocyte" in that of THÉEL. This kind of corpuscles is most abundant in the body fluid of *Molpadia*. As text-fig. 6 shows, this is a perfectly



Text-fig. 6. Hyaline amoeboid corpuscles. Vital dye-granules are seen in the cytoplasm.  $\times 900$ .

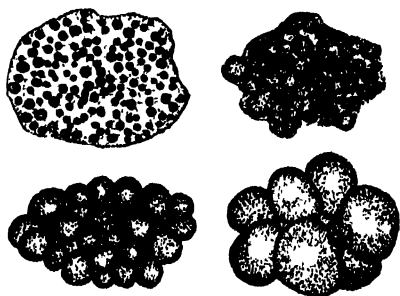
homogeneous cell, and measures  $10-18\mu$  in diameter when it takes a round shape. These are actively amoeboid, phagocytic, and show an intensive reaction to the vital staining with carmine, neutral red, trypan blue and so forth. In the cytoplasm, one or several green or brown granules are found occasionally. From the shape and size of the nucleus, these may be subdivided into two types; leucocytes and monocytes. The former has a round or oval nucleus which is situated usually in the excentrical position. The ratio of nuclear mass to that of cytoplasm is rather low. The nucleus of the latter is oval or bean-shaped, situated excentrically and occupies about half or more of the total cell volume.

In the smears of the body fluid stained with either MAY-GIEMSA's or WRIGHT's stain, hyaline amoeboid corpuscles are more or less basophilic. The nucleus stains reddish purple, and deeply basophilic coarse granular substances are visible in it. The cytoplasm is stained deep bluish and

small vacuoles are occasionally present. SCHULTZE's reaction of oxydase is intensively positive.

### 3) Colourless Granular Amoeboid Corpuscles.

This is the "amoebocyte with spherules" in the term of KINDRED and THÉEL. In the cytoplasm of the cell, colourless and coarse or fine granules are found (Text-fig. 7). The number of these granules varies



Text-fig. 7. Colourless granular amoeboid corpuscles.  $\times 1,200$ .

so remarkably that some contain only several granules while others are filled with them densely, and there exist various transitional stages between these extremes. These granules are basophilic or acidophilic and the osmic acid does not blacken them. Sometimes there exist one or several green or brown granules among colourless granules. These granules may correspond to the yellow or red granules in the leucocytes of *Arbacia punctatum*,

described and figured by KINDRED (1926). The size of cells shows great variation and  $8-20\mu$  in diameter when they are round. The nucleus is round or oval in shape and deeply basophilic. Single nucleus is found usually in the excentric position, but there exist two nuclei upon rare occasions. The lobulated polymorphic nucleus which is seen in the granulocytes of the vertebrates is not found absolutely in the present specimen. The granular amoeboid cells are less intensive than the hyaline amoeboid corpuscles in point of amoeboid movement, phagocytosis and vital staining. Oxydase reaction is also positive.

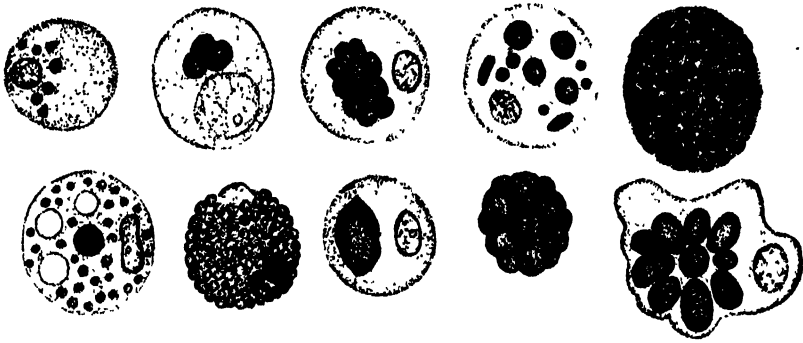
The pseudopodia of hyaline and granular amoeboid corpuscles are long and fine spine-shaped, and projecting on one side or in all directions, when the body fluid is observed *in vitro*. These are, however, petaloid or membranous, as is the case with *Caudina*, when the cells were observed after rapid fixation.

The amoeboid corpuscles of *Molpadia* show also agglutination on the withdrawal of the body fluid in a similar manner to that of *Caudina*.

### 4) Brown Amoeboid Corpuscles.

These have a cell body of clear cytoplasm in which small or large, spherical and brown bodies are embedded (Text-fig. 8). These cells are rather rare in the perivisceral fluid, but found abundantly in the water canal.

The corpuscles are actively amoeboid, phagocytic, and measure  $8-12\mu$  in diameter when they are round. They have granules which are stained with

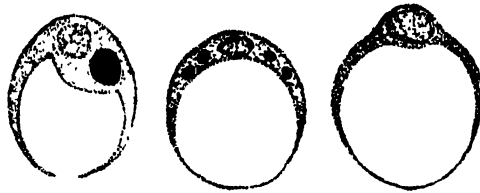


Text-fig. 8. Brown amoeboid corpuscles.  $\times 1,200$ .

neutral red *intra vitam*. The oxydase reaction is also intensely positive.

5) *Vesicular Amoeboid Corpuscles*.

These are cells, very much like the corresponding cells of the ascidian blood in appearance, and measuring about  $8-16\mu$  in diameter (Text-fig. 9).



Text-fig. 9. Vesicular amoeboid corpuscles.  $\times 1,200$ .

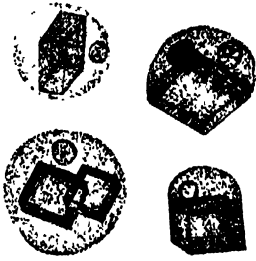
In the clear cytoplasm there is single large vacuole filled with colourless fluid. The cytoplasm is usually pressed on one side by a large vacuole. At one place the peripheral rim of cytoplasm is thickened, and here the nucleus is located. The fluid within the vacuole takes the methylen blue, neutral red or brilliant cresyl blue. In the cytoplasm usually no granule is found, but one or several green bodies occur upon rare occasions. At times these cells are seen to have filamentous pseudopodia. These cells are also oxydase-positive, but not so intensive. The vital granules of neutral red are occasionally seen in the perinuclear zone.

My knowledge of the vesicular amoeboid corpuscles of holothurid is superficial. The presence of this type of cell is reported only by THÉEL (1920-'21) in the blood of two species, viz. *Mesothuria intestinalis* and



*Psolus phantapus*, so far as I am aware. As for the general appearance of the vesicular-cells which are described and figured by THÉEL, bears close resemblance to that of the compartmental cells of ascidans. I failed, however, to detect the presence of such form in the body fluid of *Molpadia*.

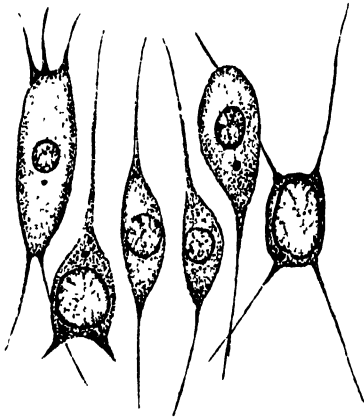
#### 6) Crystal Corpuscles.



Text-fig. 10. Crystal corpuscles.  $\times 1,200$ .

These are corpuscles, essentially similar to that of *Caudina*. In the clear cytoplasm one or two colourless rhomboidal crystal  $6-8\mu$  in long axis,  $3-5\mu$  in short axis and  $2-8\mu$  in height are found (Text-fig. 10). Single, small and round nucleus is located in the excentrical position. Crystal cells are non-amoeboïd, and show negative reaction to the vital staining. The neutral red granules are found occasionally in the preparation supravitaly stained.

#### 7) Spindle-corpuscles.



Text-fig. 11. Spindle corpuscles.  $\times 1,200$ .

These are corpuscles spindle or trigonal in shape, and measures  $6-8\mu$  is shorter diameter and  $10-16\mu$  in longer diameter (Text-fig. 11). The corpuscle has usually a large, oval or round nucleus, situated in the centre of the cytoplasm. In the clear cytoplasm, single or several green bodies are occasionally found. The spindle cells have usually long spine-like processes uni- or bipolarly. The corpuscles show active reaction to the vital and supravital staining with carmine, neutral red, trypan blue and so forth.

#### 8) Minute Corpuscles.

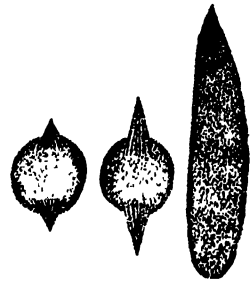
These are also essentially similar to those of *Caudina*, measuring about  $1-5\mu$  in diameter. In the hyaline cell body no structure can be seen. The corpuscle may or may not contain a single granule. After WRIGHT's stain of dry-fixed smears, the cytoplasm stains intensely with eosin. The vital and supravital staining to these corpuscles lasts constantly negative.

#### 9) Fusiform Corpuscles.

This type of corpuscles was found by THÉEL (1920-'21) in the blood of *Labidoplax*, *Thyone* and *Stichopus*, and by KAWAMOTO (1927) in that

of *Caudina*. THÉEL said that it is probably a fragement of shattered tissue to which explanation KAWAMOTO also agreed.

The fusiform corpuscles assume various forms, ranging from spherical to greatly elongated; but in the majority of cases they are shaped like a spindle. The flagellum- or pseudopodium-like prolongation of the corpuscles at one or both ends is often met with. The fusiform corpuscles vary greatly in size, measuring  $14\text{--}120\ \mu$  in length and  $6\text{--}22\ \mu$  in breadth, and every gradation in size between the extremes is seen. They consist of a thin cell membrane with distinctly fibrillous contents, which escaped the observation of THÉEL and KAWAMOTO (Text-fig. 12). The fibrills themselves are in the majority of cases arranged in a single bundle, parallel to one another, and converging toward either extremity, but in some instances there have been observed two bundles, so arranged as to form an angle with each other. The fusiform corpuscles and fibrills are stainable with eosin.



Text-fig. 12. Fusiform corpuscles.  $\times 1,200$ .

The presence of a spindle body, which are very much similar to the fusiform corpuscles, in the blood of *Phoronis* and *Lingula* is reported by many authors. FRANÇOIS (1891) observed the spindle bodies in the blood of *Lingula* at Numea. He called them 'corpusculus fusiformis', and stated that their size varies  $20\text{--}100\ \mu$  in length, and they have several striations in their bodies. In same year CORI (1891) described the spindle bodies in his anatomy and histology of *Phoronis*. In some cases, he reported, near the middle part of 'spindelförmiger Körperchen' refractive granules are found, and sometimes it is enclosed by a membrane which presents a double contour. BLOCHMANN (1900) observed the spindle bodies in the coagulated coelomic fluid of *Lingula* and positively proved the absence of the nucleus in it. YATSU (1902) made a detailed observation of the spindle bodies in the blood of *Lingula anatina* morphologically and genetically. He proved also the absence of nucleus in them, and described and figured various types of spindle bodies in respect of the arrangement of fibrills. He made clear also the development of them in the bodies of young and adult *Lingula*. He concluded that these bodies arise from the ordinary blood corpuscles. He came to discover nearly all stages of transformation from the ordinary blood corpuscles up to the spindle bodies.

What is the spindle bodies? On this problem there have been formed many hypotheses. YATSU concluded, however, from several evidences that

the spindle bodies functionate only at their formation as the eliminators of waste products. I failed to detect the transformation of blood corpuscles to fusiform corpuscles due to the degeneration of nucleus, and the formation of fibrills at the expense of metamorphosed cytoplasm, as it was observed by YATSU in *Lingula*. But it seems to be reasonable to suppose that there is a relation between the increase of fusiform corpuscles and the anemia in the blood of the animals in prolonged captivity.

#### SUMMARY

1. The coelomic corpuscles of *Molpadia roretzii* are essentially similar to those of *Caudina* except in respect of the presence of vesicular amoeboid corpuscles.

2. Hyaline, granular, vesicular and spindle corpuscles are positive to the vital staining with neutral red, brilliant cresyl blue, trypan blue &c. Crystal corpuscles are only positive when neutral red and brilliant cresyl blue were used supravitaly.

3. SCHULTZE's oxydase reaction is positive in all corpuscles except crystal, minute, and fusiform corpuscles.

4. Red corpuscles contain uni- or bipolar 'segregation apparatus', reticular patterns and mitochondria, and show positive reaction to the silver impregnation.

5. The intensive erythrocytrophagocytosis of macrophages is seen in the animals kept long in the aquarium.

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# ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES

## V. REACTION OF THE COELOMIC CORPUSCLES OF AN ECHINID, *TEMNOPLEURUS HARDWICKII* (GRAY), TO VITAL DYES AND SOME CHEMICAL REAGENTS<sup>1)</sup>

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(With six figures)

(Received May 25, 1936)

The animal here used are found abundantly in the vicinity of the Asamushi Marine Biological Station. These animals are very suitable to use for the purpose of the vital staining, for it is possible to keep them in the aquarium for several weeks fresh and vividly, and they can stand repeated injections of vital dyes with no remarkable decrease of vitality. These animals contain a large number of various granular amoebocytes. This is favourable for the study of the nature and function of these corpuscles.

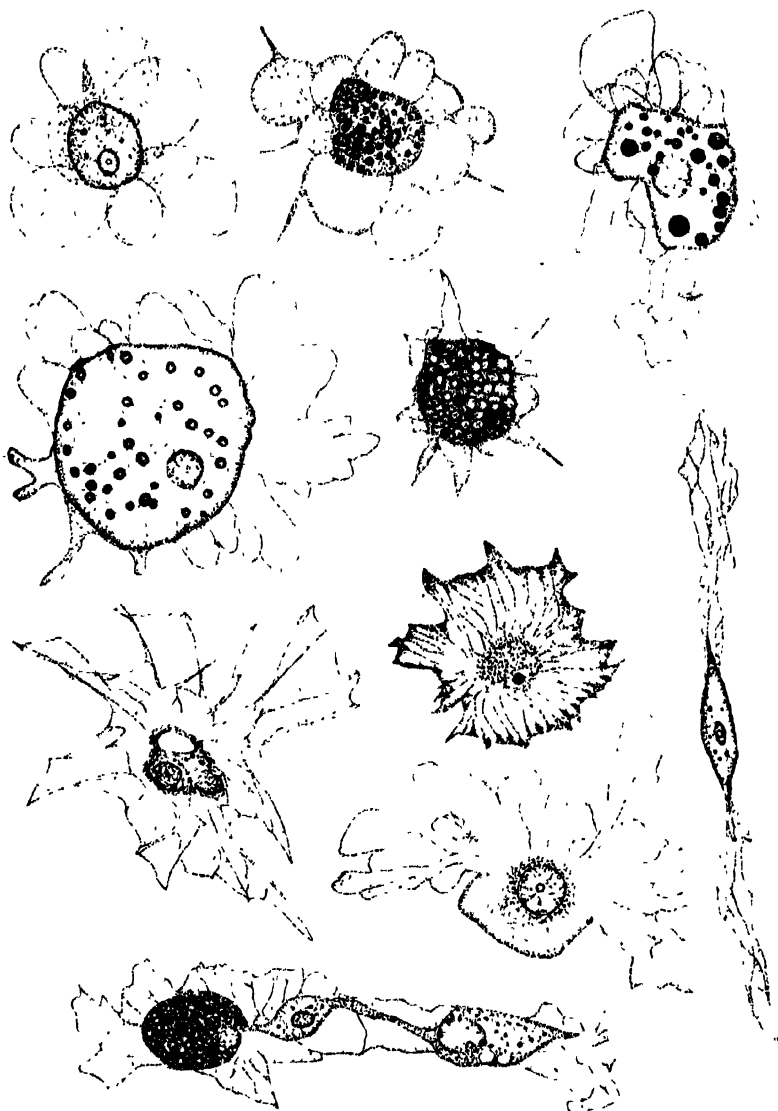
In the blood and coelomic fluid six kinds of blood corpuscles are distinguishable: 1) hyaline amoebocytes, 2) coarsely granular amoebocytes, 3) finely granular amoebocytes, 4) brown amoebocytes, 5) vesicular amoebocytes, and 6) compartmental amoebocytes.

1) Hyaline amoebocytes. This kind of corpuscle is most abundant in the body fluid of *Temnopleurus*. These are homogeneous cells, as the name shows, measuring 8–16  $\mu$  in diameter in spherical shape. The round or oval shaped nucleus is relatively small, compact and possess usually a single nucleolus in it. Occasionally one or several green or yellowish green granules are found in the cytoplasm. These cells are intensely phagocytic, and most actively react to vital staining with carmine, trypan blue, neutral red, Nile-blue sulphate and so forth. Vital double staining with carmine and trypan blue, or Nile-blue sulphate and neutral red, etc. is also possible. In these cases the proportion of both kinds of dye-granules shows a good deal of variation. Some contain only, for example, carmine granules while the other the granules of trypan blue alone, and there is seen

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<sup>1)</sup>Contribution from the Marine Biological Station, Asamushi, Aomori-ken. No. 138.

every gradation of mixtures of both kinds of dye-granules between the extremes. Supravital staining with the aforementioned dyes and others



Text-fig. 1. Various forms of hyaline amoebocytes taken from the coelomic fluid of living specimens. In the cytoplasm of some amoebocytes the vital granules of dyes are seen.  $\times 2,000$ .

such as brilliant cresyl blue, methylen blue, Janus green B, diluted GIMSA's stain etc. is also positive. The hyaline amoebocytes show a positive reaction to SCHULTZE's oxydase test while they are negative to SATO's peroxydase test. The oxydase granules are fine, nearly uniform in size, less numerous than the vital granules, and appear chiefly in the perinuclear zone. These cells are able to oxidize the Rongalit white of methylen blue and the granules of various shades between green and blue are visible in the cytoplasm.

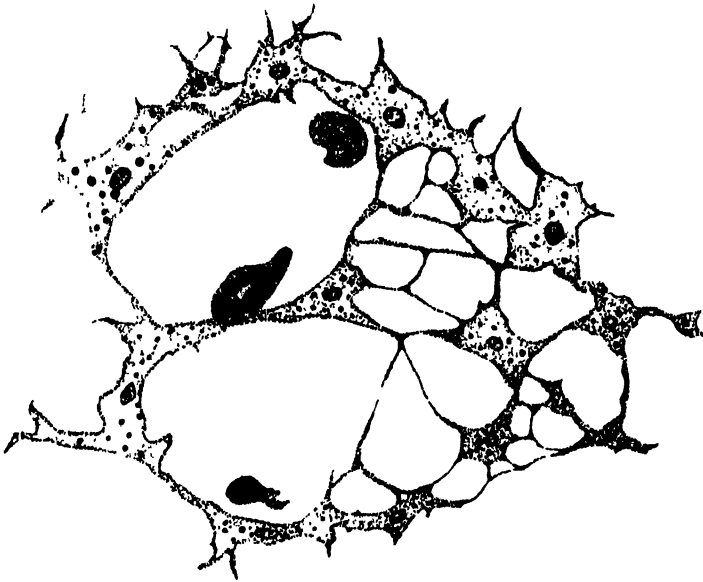
In the smears of the body fluid stained with GIEMSA'S or WRIGHT'S stain, the cytoplasm is stained deep blue, and small purple vacuoles or granules are found in it occasionally. The nucleus is stained reddish purple in colour and deeply stained granular or fibrous structure is seen usually.

When the coelomic fluid has been removed from the body of animal, the hyaline amoebocytes assume usually an asteroid form with blunt and lobular pseudopodia (Text-fig. 1). Spine-shaped pseudopodia which I found in the leucocytes of *Caudina* and *Molpadia* are not visible in the present specimens normally.

The blood corpuscles are deposited in a few minutes after removed from the animal body, and a membrane-like sediment is formed at the bottom when the body fluid is allowed to stand on a watch-glass. The coagula of blood of *Caudina* and *Molpadia* were so feeble that they are broken at once by shaking the vessel. The coagulum of the present animal is so tough that it is possible to pick it up with a forceps without breaking it. The coagulum is formed by the fusion of blood corpuscles, especially hyaline amoebocytes (Text-fig. 2). As I (1934, 1936) already reported, it is not possible to detect the real syncithium formation of blood cells in *Caudina* and *Molpadia*. In the present specimen, however, the hyaline amoebocytes fuse together with their blunt pseudopodia, forming a net-work of considerable extent. Cell-boundaries are not discernible though the coagulum is fixed with osmic acid or I-IK solution and are stained with various dyes. In the frame-work of the fused hyaline amoebocytes other kinds of blood corpuscles are found, retaining the cell-boundaries of their own distinctly. THÉEL (1920-'21) reported the syncithium formation by the fusion of many "plasma-amoebocytes" of *Labidoplax buskii*. He stated with conviction that the coelomic fluid of some echinoderms contains a fibrin-like matter which partakes in the syncithium formation. I failed, however, to detect the presence of this matter in the blood of the present specimen. Frequently the hyaline amoebocytes elongate their



own bodies to a linear shape, which reaches to  $120\mu$  or more in length (Text-fig. 1).

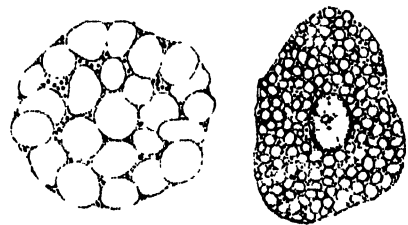


Text-fig. 2. Plasmodium formation of hyaline amoebocytes. Vital granules of trypan blue are seen in the cytoplasm of them.  $\times 800$ .

2) Coarsely granular amoebocytes. These are the corpuscles of which cytoplasm are filled up with colourless and coarse granules, and measure  $12-16\mu$  in diameter. The granules are round or oval in shape and are nearly of the same size in a given cells, measuring about  $2-4\mu$  in diameter. The nucleus is hidden with these granules and not visible on the surface. In the smears of the body fluid stained with GIEMSA's or WRIGHT's stain the granules show bluish purple in colour. The cell is negative to any vital dyes. Supravitaly brilliant cresyl blue has the most intensive affinity to the granules. With methylen blue the granules are stained metachromatically. Basophilic granules are detected by means of the supravital staining and the staining with GIEMSA's stain after fixation. Oxydase and peroxidase reactions are always negative. The granules somewhat shrink in dilute solution of acetic acid. Dilute sodium hydroxide or ammonia causes the granules to swell gradually and then to dissolve. Ether has no remarkable action on these granules. Fat-staining with Sudan III or Scharlach R. is negative the same as glycogen staining with BEST's carmine. With MILLON's reagent the granules become dirty yellow or grayish

brown in colour. In a two per cent solution of osmic acid the coarsely granular amoebocytes assume always a dark appearance. A close examination makes it clear that this darkening of the cell is due to the appearance of very fine black particles in the intergranular cytoplasm. We understand, therefore, that the granules themselves do not show any blackening with osmic acid. KOLLMANN (1908) studied the chemical nature of granules of several kinds of amoebocytes in invertebrates and stated that these granules do not react to osmic acid and are also insoluble in ether and other fat-solvents. HERDMANN and BOYCE (1899), however, reported the blackening reaction of granules to osmic acid. TAKATSUKI (1934) described that osmic acid does not produce any distinct blackening effect on the granules of the oyster amoebocytes, their margin sometimes appear blackish, but never the granules. The present observation is in accord with this description.

3) Finely granular amoebocytes, 8–12  $\mu$  in diameter. These cells are rarer than the aforementioned cells. The cytoplasm embeds a number of granules which are colourless, remarkably refractive, and smaller (about 0.5–2.0  $\mu$  in diameter) than those of coarsely granular amoebocytes. The nucleus is obscured by these granules. When these cells were stained with dilute GIEMSA's stain supravitaly, the granules become uniformly bluish purple in colour within a few minutes. A remarkable number of cells with red granules, however, appear with lapse of time, showing the presence of eosinophilic granulocytes. These cells are positive to the vital staining and indophenol reaction in contrast with coarsely granular amoebocytes. Vital and oxydase granules are few in number, very similar to each other, and appear chiefly in the intergranular cytoplasm. The preexisting granules show a very weak affinity to brilliant cresyl blue and are more resistant to alkali and distilled water than the coarse granules. These cells are also negative to Sudan III and Scharlach R., and slightly blacken by osmic acid. That this blackening of cells by osmic acid is not due to the blackening of the preexisting fine granules, but due to the appearance of very fine, black particles in the intergranular cytoplasm is equal to the case of coarse granulocytes. The fine granules



Text-fig. 3. Black particles produced by the action of osmic acid in the intergranular cytoplasm of both types of granulocytes.  $\times 1,600$ .

stained with BEST's carmine, show the presence of glycogen. Alcohol does not destroy the granules immediately, but they seem to become gradually coagulated. MILLON's reaction is negative.

From these reactions it seems to me that the fine granules have the nature of carbohydrate while coarse granules possess that of protein.

4) Brown amoebocytes, 6–18  $\mu$  in diameter. In the cytoplasm of these cells brown granules are embedded. These granules show a good deal of variation in number, namely from several to sixty or more. The size of granules is nearly uniform in a given cell, while there is seen a considerable fluctuation among different cells. These cells are phagocytic and positive to the vital staining.

The reactions of brown cells to various chemical reagents are as follows :

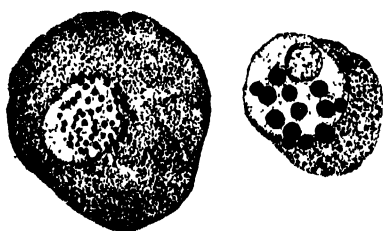
i) Acid and alkali. Brown granules are dissolved with dilute acetic acid and caustic soda. The boundaries of granules fuse out and the granules grow to a single or several large vacuoles.

ii) Alcohol and ether. These reagents dissolve the brown granules in the same manner as does acetic acid or caustic soda.

iii) Ammonia. Brown granules are dissolved in dilute ammonia and the cytoplasm turn to homogeneous brown. The nucleus which is obscured by granules becomes visible. Insoluble or hardly soluble parts of brown granules remain immediately beneath the cell membrane which is clearly distinguishable from the cytoplasm. They are more deep brown in colour, far less in number and have a more irregular contour than the original granules.

iv) Osmic acid. With osmic acid the brown granules are uniformly blackened.

v) Concentrate sulphuric acid. The granules turn to orange red in colour.



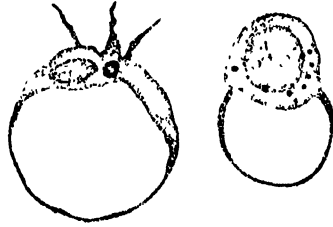
Text-fig. 4. Oxydase granules of brown amoebocytes.  $\times 1,600$ .

With these five reactions it seems to me that the brown granules of *Temnopleurus* contain a lipochrome or a pigment closely related to it.

vi) Oxydase reaction. With the action of SCHULTZE's reagent, brown granules dissolve quickly; the cytoplasm becomes brown in colour, and numerous and minute particles appear in it. A little afterwards oxydase

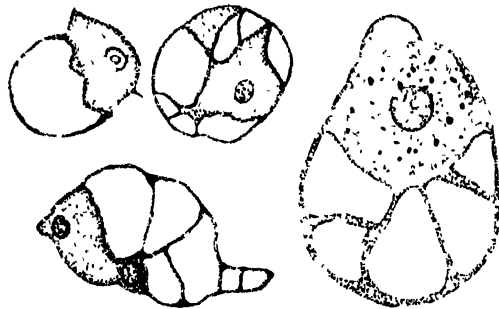
granules appear in the perinuclear zone. They are numerous and nearly uniform in size within a given cell (Text-fig. 4).

5) Vesicular amoebocytes. These are quite like the corresponding cells of ascidian blood and young linocytes of earthworm in appearance, and measure approximately  $10-18\mu$  in diameter (Text-fig. 5). These cells are positive to oxydase reaction and vital staining. Occasionally needle-like pseudopodia are visible. Colourless fluid filling the vacuole is stained with neutral red, methylen blue or brilliant cresyl blue. Dancing particles are not visible in it of any specimen.



Text-fig. 5. Vesicular amoebocytes taken from a living specimen.  $\times 1,600$ .

6) Compartmental amoebocytes,  $8-18\mu$  in diameter. These are also essentially like those of the ascidian blood. They are colourless and have a variable number of large, round or somewhat angular vacuoles, filled with colourless fluid. The fluid is stainable vitally with



Text-fig. 6. Compartmental amoebocytes.  $\times 1,600$ .

neutral red, brilliant cresyl blue, and so forth. In the cytoplasm, no granules are usually visible, but very minute particles are found when the cell was fixed with osmic acid. The compartmental amoebocytes are also positive to the oxydase reaction and the vital staining. They send forth occasionally blunt pseudopodia.

#### SUMMARY

In the blood and coelomic fluid of *Temnopleurus hardwickii*, six kinds of blood corpuscles are distinguishable. These are all positive to the vital staining and the oxydase reaction with the exception of that of per-oxydase. The fine granules of granulocytes have the nature of carbohydrate while the coarse granules possess that of protein. In the brown amoebocytes a kind of lipochromes is contained. The plasmodia are formed by the fusion of hyaline amoebocytes.

## ACKNOWLEDGEMENT

It is a pleasure to express my hearty thanks to Dr. S. HATAI for his kindness in reading and criticizing the manuscript in spite of his precious time. I am also indebted to Assist. Prof. I. MOTOMURA for his valuable suggestion and advice during the course of the work.

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# ON THE COELOMIC CORPUSCLES IN THE BODY FLUID OF SOME INVERTEBRATES

## VI. A NOTE ON THE FORMED ELEMENTS IN THE COELOMIC FLUID OF A BRACHIOPOD, *TEREBRATALIA COREANICA*<sup>1)</sup>

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(With Plate IV and one text-figure)

(Received May 25, 1936)

The formed elements of the coelomic fluid of *Brachiopoda* drew much less attention of the comparative haematologists. Even in the exhaustive investigations of CUÉNOT (1891), and KOLLMANN (1908) on the blood of invertebrates there is no description on this subject. What we know of the histology of the formed elements of body fluid of *Brachiopoda*, we owe chiefly to the studies of FRANÇOIS (1891), BLOCHMANN (1900), YATSU (1902) and few others. Material used by these authors is limited to the animals of genus *Lingula* which belong to order *Ecardines*. As to the animals belonging to order *Testicardines*, therefore, the histology of coelomic corpuscles still remains nearly in the dark, so far as I am aware. The present paper is believed, superficially and fragmental though it be, worthy of publishing, as it may help to throw some light upon the subject in question.

I made observation on the body fluid of a species, *Terebratalia coreanica* ADAMS et REEVE, which is abundant in the Mutsu Bay, and I collected them exclusively in the vicinity of the Asamushi Marine Biological Station.

Before going further I should like to express my gratitude to Dr. S. HATAI for the help and advice he has so kindly given me.

In the coelomic fluid of *Terebratalia*, we find six kinds of formed elements: 1) hyaline amoebocytes, 2) coarsely granular amoebocytes, 3) finely granular amoebocytes, 4) amoebocytes with red granules, 5) amoebocytes with orange granules and 6) amoebocytes with brown granules.

1) Hyaline Amoebocytes. The coelomic fluid which fills up the body cavity is found as sparsely scattered cells in which the hyaline amoebocytes

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<sup>1)</sup>Contribution from the Marine Biological Station, Asamushi, Aomori-Ken. No. 139.

occupy a chief part. Hyaline amoebocytes are colourless, transparent, and spherical in form when they are at rest, and measure 8 to 12  $\mu$  in diameter (Plate IV, fig. 1). These cells are positive to the vital staining, supravital staining (Plate IV, figs. 4-7) and oxydase reaction. The oxydase granules are usually similar to the supravital granules of neutral red, methylen blue, Nile-blue sulphate, brilliant cresyl blue &c., and somewhat smaller and less numerous than the vital granules of carmine and trypan blue. In the smears of body fluid stained with GIEMSA's dye the cytoplasm stains homogeneously blue or purplish blue, but occasionally very fine and azurophilic granules are met with in the perinuclear zone (Plate IV, figs. 2 and 3). The nucleus is usually single in a given cell. It is relatively small and compact, and possesses a nucleolus in the majority of cases. The nucleus of monocyte-type is not found in any specimen.

Hyaline amoebocytes are phagocytic and show a tendency to agglutinate into a mass by fusing each other with their pseudopodia.

YATSU (1902) described the 'blood corpuscles' of *Lingula*. These are one of three formed elements in the coelomic fluid and are colourless, hyaline and basophilic cells, measuring 10-20  $\mu$  in diameter. These are, perhaps, the cells corresponding to the hyaline amoebocyte of *Terebratalia*.

2) Coarsely Granular Amoebocytes. These are found in a small number in the coelomic fluid, and measure 12 to 20  $\mu$  in diameter. The cytoplasm is filled up with colourless, coarse granules which are usually of the same size (2-4  $\mu$  in diameter) in a given cell, but sometimes large granules are present.

The coarsely granular amoebocytes are distinguishable into two subtypes according to the chromatic properties of granules. One of them is a cell which contains purple (basophilic) granules when it is fixed and stained with GIEMSA's or WRIGHT's stain (Plate IV, figs. 12 and 13). These granules are intensely positive to the supravital staining with brilliant cresyl blue, positive but less intensive to that with neutral red, methylen blue, methyl violet &c. (Plate IV, figs. 8-10). This type of cell is also positive to oxydase reaction; when SCHULTZE's reagent is applied, numerous blue specks appear on the surface of each granule. Another type of cell is filled with acidophilic granules which are more highly refractive than the former, and negative to supravital staining with brilliant cresyl blue while they show a positive reaction to neutral red and trypan blue (Plate IV, fig. 11). In the majority of cases the coarse granulocytes are negative or slightly positive to the vital staining. The vital granules of trypan blue appear among the preexisting colourless granules and vary

in size and number. The nucleus is spherical and relatively small, and it is usually obscured by granules. Amoeboid movement, phagocytosis and agglutination are less active than in the first formed element.

The granules are negative to Sudan III and Scharlach R. They are not blackened with osmic acid and not soluble in fat-solvents. Distilled water does not destroy them. In dilute acetic acid they seem to become gradually coagulated. Dilute alkali causes the granules to swell gradually and then to dissolve. No positive reaction is visible in apply MILLON's reagent.

3) Finely Granular Amoebocytes. These are also an infrequent element of coelomic fluid. They measure 10-18  $\mu$  in diameter when they take a round shape. The granules which fill up the cytoplasm are smaller in size (1-3  $\mu$  in diameter) and more highly refractive than those of the coarsely granular amoebocytes. They are amphophilic or acidophilic in the smear preparation stained with GIEMSA's or WRIGHT's stain (Plate IV, figs. 14 and 15). Supravivally some are very intensely positive to brilliant cresyl blue while some others are almost negative to that dye. To neutral red and trypan blue they have an affinity of various degrees. Positive reactions are obtained with SCHULTZE's reagent and various vital dyes. The granules behave similarly to those of the coarsely granular amoebocytes to Sudan III, Scharlach R., osmic acid and other chemical reagents. The amoeboid movement is more active than in the coarsely granulated type of cells and few blunt pseudopodia are sent forth from ectoplasm.

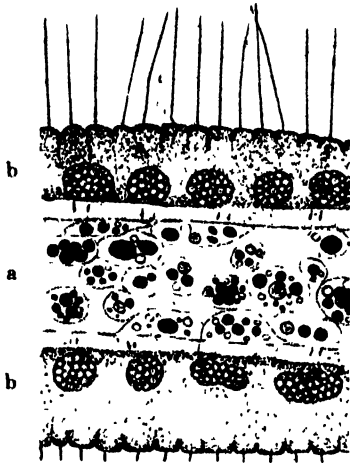
YATSU described 'leucocyte' as the second element of the body fluid of *Lingula*. This is amoeboid and filled up with fine granules which are of acidophilic nature. This type of cell is, therefore, perhaps the one which corresponds to the finely granular amoebocyte in the present case.

4) Amoebocytes with Red Granules. These cells are infrequent in the coelomic fluid while they are present in a remarkable number in the blood vessels.

Innumerable cirri are, of course, found on a margin of arm apparatus, and they are suitable material for demonstrating red amoebocytes. For there are found many cells of this kind in the axial vessel of every cirrus and the cirri are so fine that it is possible to examine their structure and contents under oil-immersion *in situ*. The size of cells varies remarkably and measures 6-18  $\mu$  in diameter (Text-fig. 1). The cytoplasm embeds the granules which are vermilion or cherry red in colour; hence the name. The size of granules fluctuates between 2-6  $\mu$  in diameter, and from one to six is the usual number in a single cell, but those cells are found



occasionally in which a dozen or more of granules are conglomerated like a mulberry-formed packet. Besides these red granules the cells contain



Text-fig. 1. Optical section of a cirrus.  $\times 600$ . a. Amoebocytes with red granules. b. Amoebocytes with brown granules.

colourless granules similar to those of coarsely granular amoebocytes in appearance and size. The colourless granules are less numerous in the majority of cases than the red granules in a given cell, and such a small (perhaps young) cell as contains only a single red granule in it is frequently seen. In the living cells a clear area of cytoplasm which contains usually a single nucleus is distinguishable. The nucleus is vesicular and one or two nucleoli are found in it.

In the smear preparation of blood, stained with GIEMSA's stain, the red granules remain unstained, while the nucleus stains reddish purple, cytoplasm and colourless granules blue or purplish blue (Plate IV, figs. 16-22). Supravitaly

the colourless granules are stainable with brilliant cresyl blue, neutral red, methylen blue &c. while the red granules are only stained with brilliant cresyl blue. Vital granules of brilliant cresyl blue are found in these cells of some animals which received previously repeated injections of this dye.

The red granular amoebocytes are more or less amoeboid, putting forth a few blunt pseudopodia.

I made some observations on the chemical properties of red granules.

i) Fat-solvents. The red pigment is easily soluble in fat-solvents such as alcohol, ether, chloroform, benzol and so forth. The red pigment remains diffusely distributed in the cell for a time and then fades out entirely, leaving a reticulated or vacuolated cell with an excentrically placed nucleus and colourless granules.

ii) Distilled water. In the distilled water, the cells swell gradually and are destroyed within a few minutes. The granules of these cells are also destroyed and the pigment is dissolved in water. The pigment is more easily soluble in hot-water.

iii) Diluted acids and alkali solution. The diluted solution of hydro-

chloric acid and acetic acid destroys the granules. Diluted sodium hydroxide causes the granules to swell gradually and then to dissolve. The dissolved pigment is bright red in acid solution and yellowish red in diluted caustic soda. The dissolution of red pigmented granules may occur practically instantaneously or more slowly, according to the strength of acid or alkali reaching the cell. In case where acid or alkali acts more slowly, it is possible to observe the red pigment begin to diffuse and the granules disappear one by one.

iv) Concentrated sulphuric acid. Colour reaction to this reagent which is seen in the case of carotene &c. does not take place in this pigment.

v) Ammonia. There is no change in granules or pigment in a diluted solution of ammonia.

vi) Osmic acid. The pigment is dissolved in 2 per cent solution of osmic acid without any colour change.

vii) Hydrogen peroxide. The bleaching of colour does not occur with hydrogen peroxide.

That the present pigment is not one of lipochrome is evident from the reactions to water, osmic acid and sulphuric acid. It seems to be presumable also from its colour and solubility that this pigment does not belong to any of haemoglobin, haematin, haematoporphyrin or haemerythrin. Vanadium compounds found in the ascidian blood corpuscles show occasionally orange or orange red colour, but this dye is turned to blue by the action of reducing agents, such as alcohol &c. I suppose, therefore, that this pigment may be a kind of echinochromes (MAC MUNN 1885) which is soluble in water and fat-solvents and becomes orange or yellow when it is treated with caustic soda. But I will refrain from assertion on this point, for I have made no spectroscopic examination which is indispensable to the investigation of blood pigment.

According to MAC MUNN, the echinochrome is a respiratory pigment. If the pigment in question is useful for respiration the structure of the cirrus is in accord with this purpose very well. Every cirrus is provided with long cilia on its surface, and the current of coelomic fluid is brought about by the oscillation of these cilia. The cirri, therefore, receive an ample supply of oxygen. This oxygen may combine with the pigment of red amoebocytes and be carried somewhere by blood current.

To my knowledge the occurrence of red pigment other than lipochromes and vanadium compound in the leucocytes is nearly restricted to the class *Echinoidea*. It is of interest that a red pigment in question is found very abundantly in the leucocytes of *Brachinopoda*.

5) Amoebocytes with Orange Granules. Of the fifth element, the orange amoebocytes, a small number are found in the coelomic fluid, while a considerable number exist in the blood vessels (Plate IV, figs. 23-25). They measure  $10-18\mu$  in diameter when they take the round shape. These cells have a cell body of clear cytoplasm which embeds a considerable number of spherical orange-coloured bodies. Besides these granules there are found usually several colourless granules. The small and few green granules are seen simultaneously therein upon rare occasions. As in the case of red amoebocytes, there is an excentric mass of cytoplasm where the vesicular nucleus is located. The nature of colourless granules is similar to that of the cell mentioned just above. The orange granules are blackened with osmic acid. They turn greenish blue with SCHULTZE's reagent, showing the presence of oxydase in them, but these granules are not disintegrated into small particles as was the case in the brown corpuscles of *Temnopleurus*. The orange amoebocytes show relatively active amoeboid movement and have a tendency to agglutinate with other kinds of amoebocytes.

6) Amoebocytes with Brown Granules. These cells are scattered in abundance in the cirri and on the surface of the coelomic membrane while they are met with but seldom in the coelomic fluid (Plate IV, figs. 26-29). These measure  $8-16\mu$  in diameter. In point of structure they are very similar to the finely granular amoebocytes, but the one type of cells are distinguishable from the other in the other various properties. These cells consist of yellowish gray-coloured granules embedded in clear cytoplasm. The nucleus is hidden by these granules and usually invisible on the surface. In the cirri these cells are found in the subepithelial tissue which surrounds the axial vessel, and are arranged usually in a row with regular intervals. The brown cells, therefore, stand in juxta-position to the red amoebocytes in the axial blood vessel, intervened by a thin wall which is transparent, elastic and so resistant that it remains unchanged in distilled water, alcohol, diluted acids or alkali. That these cells are not fixed in tissue is evident as they move slowly in the intracellular space, giving off lobular pseudopodia. During this movement clear ectoplasm stretches out in one direction at first, and then granular endoplasm flows into it, as is seen in the usual amoeboid movement. Occasionally they become so slender on account of the elongation that their length exceeds the breadth by five or six times. The granules are usually of the same size in a given cell and amphophilic with more or less basophilic inclination. When these granules are examined after supravital staining

with methylen blue, brilliant cresyl blue and so forth we can recognize that the granules contain many small dancing particles which are intensely stained with these dyes. From this fact it is evident that the granules consist of liquid substance. The granules are blackened with osmic acid, but negative to Sudan III and Scharlach R. Intensive indophenol synthesis is visible in every granule, and the aforementioned fine dancing particles are stained intensely by this reagent.

As it was reported by some authors (FRANÇOIS 1891, BLOCHMANN 1900, YATSU 1902 &c.) the spindle body is a common element in the body fluid of *Lingula*. In the present case, however, the presence of this type of corpuscle is yet in question.

#### SUMMARY

Six kinds of formed elements are distinguishable in the body fluid of *Terebratalia coreanica*. Among these the occurrence of the amoebocyte with red granules are most interesting. It is supposed that the red pigment of these cells may have a close relation with the echinochrome, considering some of their chemical properties. As for the orange and yellow-gray granules, I believe these are a kind of lipochromes.

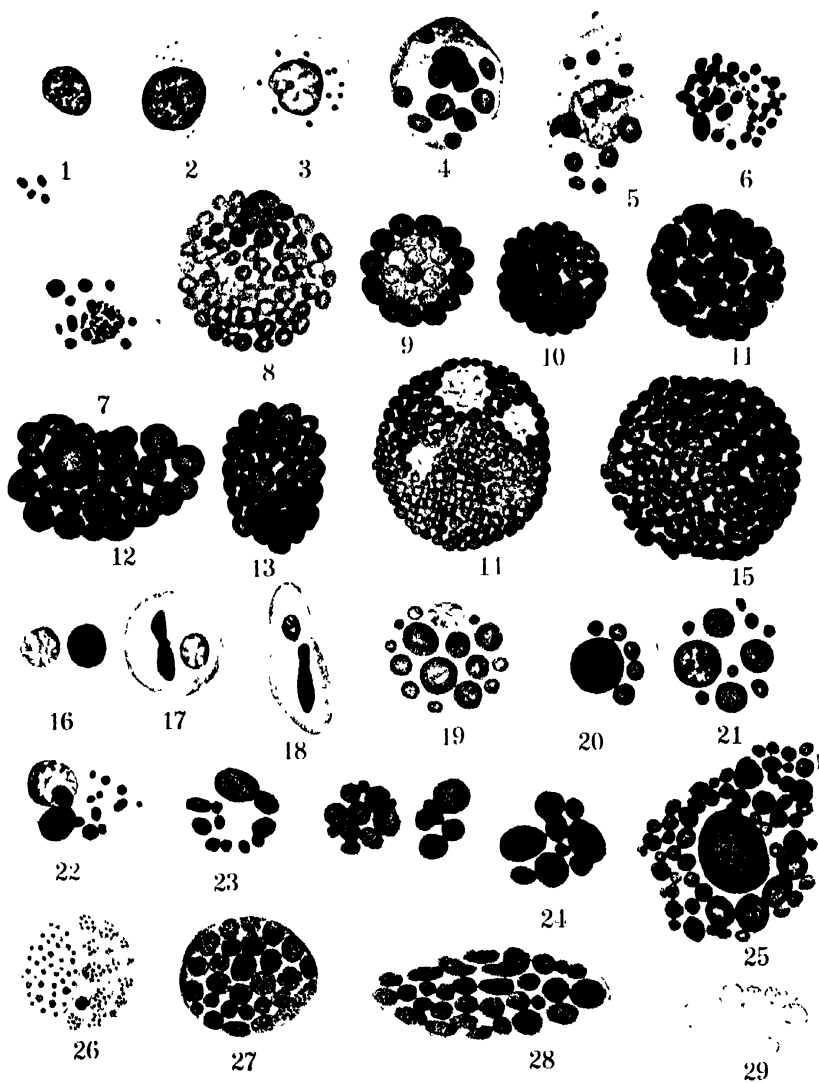
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## EXPLANATION OF PLATE IV

All figures were drawn with the aid of a camera lucida at a magnification of 1,600 diameters.

- 1- 3, Hyaline amoebocytes from the smear of coelomic fluid. In figs. 2 and 3 the azurophilic granules are seen. GIEMSA.
- 4- 7, Hyaline amoebocytes with the vital dye granules taken from living specimens which received the injections of trypan blue or neutral red, or both of these dyes. In these figures, 4 and 5 are cells with granules of trypan blue; 6, cell with those of neutral red and trypan blue; 7, cell with those of neutral red. The nucleus and cytoplasm were stained with methylen blue after the formalin-fixation.
- 8-11, Vital staining of the coarsely granular amoebocytes. The cells were stained with brilliant cresyl blue (8), trypan blue (9 and 10), or neutral red (11) *infra vitam*.
- 12-13, Coarsely granular amoebocytes. Smear. GIEMSA.
- 16-22, Amoebocytes with red granules, fixed with formalin and stained with methylen blue.
- 23-25, Amoebocytes with orange granules, supravitaly stained with concentrated solution of brilliant cresyl blue.
- 26-29, Amoebocytes with brown granules. Fresh and unstained.



Author del.



# KERNPHASENWECHSEL VON *HETEROCHORDARIA ABIETINA*<sup>1)</sup>

VON

KÔGORÔ ABE

*Biologisches Institut der Kaiserlichen Tôhoku Universität, Sendai*

(Mit Tafeln V-VI)

(Eingegangen am 6. Juli 1936)

Bei *Heterochordaria abietina* (RUPR.) SETCH. et GARDN., eine Spezies der Heterochordariaceen, findet man uni- und plurilokuläre Sporangien in getrennten Individuen. In meiner letzten Mitteilung ('35) über diese Pflanze, machte ich Vorstellung, dass die Individuen mit unilokulären Sporangien diploid und die mit plurilokulären haploid sein sollten. Um dies zytologisch genau festzustellen, unternahm ich wieder die vorliegende Arbeit. Material hierfür wurde in der Biologischen Station zu Asamushi gesammelt.

Als Fixierungsmittel benutzte ich dieselbe Lösung, welche ich bei meiner Untersuchung über die Mitosen im Antheridium von *Sargassum confusum* ('33) mit gutem Erfolg gebraucht hatte. Das Material blieb in dieser Lösung gewöhnlich 1.5–2 Stunden. Die Ergebnisse waren meistens trefflich. Die 5–6  $\mu$  dick geschnittenen Paraffinschnitte wurden mit HEIDENHAIN's Eisenalaunhämatoxylin gefärbt.

Fig. 1 zeigt den sich im vollständigen Ruhestadium befindlichen Kern des unilokulären Sporangiums. In diesem Stadium beträgt der Durchmesser des Kerns etwa 3–4  $\mu$ . In Figg. 3–4 sieht man ein Synapsisstadium. Darauf folgt das Spirem- und Diakinesestadium (Figg. 5–9). In Fig. 9 ist der Nukleolus schon verschwunden. Bei solchen Stadien konnte ich feststellen, dass die Zahl der Chromosomen mit grosser Wahrscheinlichkeit 20 beträgt. Vor der Auflösung der Kernmembran ordnen sich die Chromosomen allmählich auf der Äquatorialebene an (Figg. 10–12). Die vollständige Metaphase erfolgt aber nach Auflösung der Kernmembran. Fig. 14 gibt dasselbe Stadium in Polansicht wieder, wobei sich auch die etwa 20 Chromosomen zählen lassen. In der Seitenansicht der Spindel ist ein zentrosomähnliches Körperchen in jedem Pole sichtbar (Fig. 13). Die Anaphase geht normal vor sich (Fig. 15). In der Telophase berühren

<sup>1)</sup> Contribution from the Marine Biological Station, Asamushi, Aomori-Ken, No. 140.



sich die beiden Tochterkerne eng aneinander (Fig. 16). Nach kurzer Pause beginnt die homöotype Teilung. Nach dieser Teilung entstehen natürlich vier Kerne, die dann noch weitere Teilungen ausführen. Inzwischen vergrössert sich das Sporangium allmählich (Figg. 17–18). Das Protoplasma teilt sich dann auf; jede Portion enthält einen Kern und einen oder zwei Chromatophoren und entwickeln sich später zu Sporen (Figg. 19–21).

Figg. 22–23 zeigen die Metaphase der ersten Kernteilung der Paraphysenzelle vom Individuum mit unilokulärem Sporangium. In der Polansicht einer solchen Kernplatte konnte ich feststellen, dass die Zahl der Chromosomen etwa 40 beträgt. Sie ist somit doppelt so gross wie im Kerne des Sporangiums. Figg. 24 und 25 zeigen Pro- und Metaphasestadium der Kernteilung in Rindenzellen von demselben Individuum. In der Metaphase der ersten Kernteilung in der Paraphysenzelle des Individuums mit plurilokulärem Sporangium konnte ich ungefähr 20 Chromosomen feststellen (Fig. 27). Ein zentrosomähnliches Körperchen ist auch in diesem Falle sichtbar. Wie aus Figg. 24 und 26 zu ersehen ist, ist die Spindel des unilokulären Individuums etwas grösser als die des plurilokulären.

Aus obigen Resultaten ist es sicher, dass die Individuen mit unilokulären Sporangien diploid, die mit plurilokulären haploid sind, und die erste Kernteilung des unilokulären Sporangiums eine Reduktionsteilung ist. Diese Pflanze zeigt also gewöhnlich einen regelmässigen Generationswechsel auf. Aber wenn die Schwärmer aus unilokulärem Sporangium nach Kopulation und die aus plurilokulärem ungeschlechtlich keimen, wie ich schon früher beobachtet habe ('35), wird der Generationswechsel unterdrückt.

Zum Schluss sei es mir gestattet, Herrn Prof. Dr. M. TAHARA, unter dessen Leitung vorliegende Arbeit entstanden ist, meinen besten Dank auszusprechen.

#### LITERATURVERZEICHNIS.

- 1) ABE, K., 1933. Mitosen im Antheridium von *Sargassum confusum*. Sci. Rep. Tōhoku Imp. Univ., Biol., Vol. VIII, pp. 259–262.
- 2) ABE, K., 1935. Zur Kenntnis der Entwicklungsgeschichte von *Heterochordaria*, *Scytosiphon* und *Sorocarpus*. Sci. Rep. Tōhoku Imp. Univ., Biol., Vol. IX, pp. 329–337.
- 3) ABE, K., 1935. Kopulation der Schwärmer aus unilokulärem Sporangium von *Heterochordaria abietina*. Sci. Rep. Tōhoku Imp. Univ., Biol., Vol. X, pp. 287–290.

## TAFELERKLÄRUNG.

Alle Figuren wurden mit Hilfe eines Anné'schen Zeichenapparatus gezeichnet, unter Benützung des ZEISS'schen Objektiv, Ölimmersion 1/12, des ZEISS'schen Okular  $\times 17$  und des LEITZ'schen Okular  $\times 4$ . Figg. 1-18 und 22-23  $\times 3100$ , Figg. 19-21  $\times 1750$ .

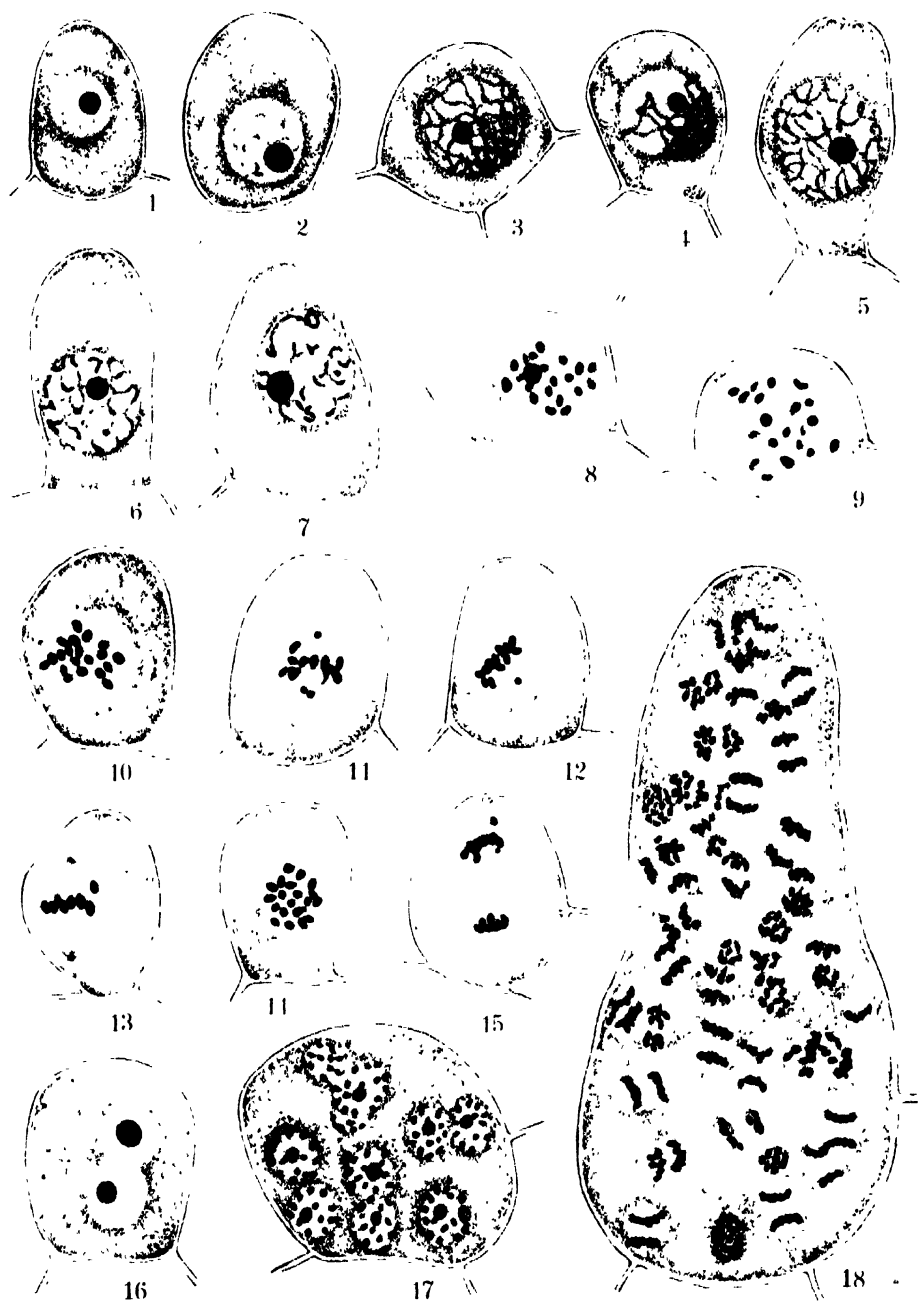
## TAFEL V.

- Figg. 1-2. Ruhestadium.  
Figg. 3-4. Synapsis.  
Figg. 5-6. Diakinese.  
Figg. 10-12. Frühere Metaphase.  
Fig. 13. Vollständige Metaphase in Seitenansicht  
Fig. 14. Dieselbe in Polansicht.  
Fig. 15. Anaphase.  
Fig. 16. Telophase.  
Figg. 17-18. Vielkernige Stadien.

## TAFEL VI.

- Fig. 19. Vielkerniges Stadium.  
Fig. 20. Fast reifes, unilokuläres Sporangium.  
Fig. 21. Voll reifes, unilokuläres Sporangium.  
Fig. 22. Metaphase der ersten Kernteilung der Paraphysenzelle vom Individuum mit unilokulärem Sporangium in Seitenansicht.  
Fig. 23. Dieselbe in Polansicht.  
Fig. 24. Metaphase der Kernteilung in Rindenzelle von demselben Individuum in Seitenansicht.  
Fig. 25. Prophase derselben Kernteilung.  
Fig. 26. Metaphase der ersten Kernteilung in Paraphysenzelle des Individuums mit plurilokulärem Sporangium in Seitenansicht.  
Fig. 27. Dieselbe in Polansicht.





K. ABE: Kernphasenwechsel von Heterochordaria.





K. ABE: Kernphasenwechsel von Heterochordaria.



## SYMBOLAE ITEOLOGICAE II

AUCTORE

ARIKA KIMURA

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(Cum 2 tabulis et 3 figuris in textu)

(Opus acceptum 11 Jul. 1936)

- 10)  $\times$  *Salix Turumatii* KIMURA hybr. nov. (Fig. 1 & Tab. VII & VIII).  
= ? *Salix Bakko*  $\times$  *S. futura*  $\times$  *S. gracilistyla*.

*Frutex.* *Ramuli* annotini elongati graciles luteo-brunnei laeves, partibus supra nodos exceptis glaberrimi, basi circ. 5 mm superne circ. 2 mm crassi; hornotini folia adulta gerentes in sicco sordide lutescentes ad fusci, minutissime pubescentes, novelli vernaes sericei. *Gemmae* amentiferae ovato-ellipsoidales, latere angulatae, apice obtusae et subrostratae, colore castaneo-brunneae, fere glabrae vel partim pulverulento-pubescentes, 1.7 cm longae; foliiferae ovatae obtusae luteo-brunneae 5–6 mm longae. *Cataphylla* steriliū ramulorum subsessilia vel brevissime petiolata, elliptica vel ovata, apice obtusa, margine integerrima, supra glabra subtus villosa, villis marginem superantibus, infima 7–9  $\times$  3.0–4.0 mm etc. magna. *Folia recentissima* sub vernatione convoluta densissime villosa, e vernatione relaxata in margine infero revoluta deinde plana. *Folia adulta* chartacea interstitiis 1–2.8 cm longis dissita, oblonga medio latiora, apice breviter acuminata, basi margine arcuato-convexo late acuta, margine glanduloso-crenato-serrata, serraturis in medio folii 2–3 et prope apicem 7–9 pro 1 cm, basi obsoletis, supra sat viridia paullum nitentia, glaberrima (costa excepta), subtus glauca pilis albis molliter sericea, 9–11 cm longa 3–3.7 cm lata, plerumque 2.5–3-plo longiora quam latiora; costa straminea, supra in sicco convexa vel planiuscula, per totam longitudinem minutissime pubescente vel apicem versus glabrescente, subtus prominente dense sericea; nervis primariis pallidis arcuatis, ante marginem leviter flexuosis, utrinque 7–12 a costa sub angulis 40°–60° divergentibus, supra paullum elevatis infra prominentibus, secundariis tenuibus et crebris, infra elevatis, inter primarios transversis fere modo *S. gracilistylae* vel *S. gracilistylويدis*, intermediis 0–2. *Petioles* supra leviter convexi ad basin concavi, sordide velutino-pubescentes, subtus convexi etiam pubescentes denique glabrescentes, ad 1.9 cm longi. *Stipulae* semicordatae apice acuminatae, dentatae, supra parce pubescentes basi



glanduliferae, subtus glaucae minute adpresseque pubescentes, 4–8.5 mm longae 1.7–4 mm latae. *Amenta* ♂ praecocia densiflora, rhachidibus sericeis invisibilibus, oblongo-cylindrica, apice rotundata basi sessilia, ante anthesin villosissima, 3–5 cm longa et ad 2 cm crassa, basi cataphyllis 2–5 squamosis viridibus, elongato-ovatis, sessilibus integerrimis apice obtusis, supra glabris infra villosis, 4–6 mm longis 2–4 mm latis suffulta. *Bracteolae* oblanceolato-oblongae, circ. 2.8 mm longae 1 mm latae, circ.  $\frac{1}{4}$  sub apice latissimae, inde sursum acutatae et deorsum subcuneatae, ad summum obtusae vel acutae, dimidia superiore parte nigrescentes, inferiore pallidae, utrinque villosissimae. *Glandula* una ventralis linearis apice truncata 1.2 mm longa 0.3 mm lata. *Stamina* 2, filamentis tantum ima basi vel e basi ad  $\frac{1}{4}$  vel  $\frac{1}{3}$  usque connatis, basi paucipilosis 8–9 mm longis. *Antherae* luteae ovatae circ. 1 mm longae. Planta feminea nobis ignota.



Fig. 1. *Salix Turumatis* KIMURA.

A Flos ♂.

B Basis filamentorum.

HAB. JAPONIA. *Honsyū*.—Prov. Hitati: Midoriga-okamura, (TURUMATI ♂ fl. [Typus] 29. Mart. 1935 in Herb. A. KIMURA, fl. 19 Apr. 1934, fol. 27 Maio 1934, 25 Sept. 1934, 5 Aug. 1935.—Omnia specimina ex eadem stirpe lecta).

Ad *S. leucopithecia* (= *S. Bakko* × *S. gracilistylam*) proxima, distat tamen ab ea ramulis lutescentibus (non purpurascentibus), cataphyllis amentorum obtusis (non acutis), foliis adultis basi acutis (non rotundatis nec cordatis), glandula ventrali lineari (non oblonga) et filamentis basi pilosis. Probabiliter hybrida inter *S. leucopithecia* et *S. futuram*, ad quam vergit planta nostra praecipue ramulis lutescentibus, foliis adultis ad costam nervosque pilis longis sericeis. Pilositas filamentorum autem ab *S. Bakko* derivata esset.—Nomen in honorem collectoris TURUMATI ill. dedi.

11) *Salix futura* SEEMEN<sup>1)</sup>, Salic. Jap. p. 71, t. 17, fig. F–G<sup>1</sup> (1903).—SCHNEIDER in SARGENT, Pl. Wilson. III. p. 177 (1916).—MATSUMURA, Ind. Pl. Jap. II. 2, p. 9 (1912).—MAKINO & NEMOTO, Fl. Jap. ed. 1, p. 1123 (1925); ed. 2, p. 164 (1931).—NEMOTO, Fl. Jap. Suppl. p. 107 (1936).

<sup>1)</sup>Vidi clastotypum (i.e. fragmenta amenti et cataphylla nonnulla) in Herbario Universitatis Imperialis Kyotensis asservatum.

· Syn. *Salix vulpinoides* KOIDZUMI in Tokyo Bot. Mag. XXVII. p. 94 (1913).—SCHNEIDER in SARGENT, Pl. Wilson. III. p. 157 (1916).—MATSUMURA, Shokubutsu-Mei-I, ed. 9, p. 355 (1916).—MAKINO & NEMOTO, Fl. Jap. ed. 1, p. 1131 (1925); ed. 2, p. 174 (1931).—NEMOTO, Fl. Jap. Suppl. 118 (1936). Syn. nov.

*Salix vulpina* ANDERSSON var. *tomentosa* KOIDZUMI in Tokyo Bot. Mag. XXVII. p. 265 (1913); ibid. XXVIII. p. 285 (1914).—SCHNEIDER in SARGENT, Pl. Wilson. III. p. 131 (1916) in adnota ad *S. vulpinam* var. *coriaceam* KOIDZUMI.—MAKINO & NEMOTO, Fl. Jap. ed. 1, p. 1131 (1925); ed. 2, p. 174 (1931).—NEMOTO, Fl. Jap. Suppl. p. 118 (1936). Syn. nov.

*Salix vulpinoides* KOIDZUMI var. *tomentosa* KOIDZUMI ex MATSUMURA, Shokubutsu-Mei-I, ed. 9, p. 355 (1916). Syn. nov.

NOM. JAP. *Ônekoyanagi* KOIDZUMI in Tokyo Bot. Mag. XXVII. p. 95 (1913).—*Okituneyanagi* KOIDZUMI ibid. XXVIII. p. 286 (1914).

HAB. JAPONIA. *Honsyû*.—Prov. **Wakasa**: Amagoiyama, (Z. TASIRO fol. 10 Jul. 1932 HK<sup>1)</sup>).—Prov. **Etizen**: Rokurosi, pede montis Kyogadake, (T. HONDA ♀ fl. 7 Maio 1934 HK); Kazatanitôge, (Z. TASIRO ♂ defl. 29 Maio 1932 HK); Ihuriyama, (Z. TASIRO fol. 13 Sept. 1931 HK); monte Yasyagatake, (Z. TASIRO fol. 6 Oct. 1929 HK); Monzyusan, (Y. HORI fol. 15 Oct. 1928 HK).—Prov. **Kaga**: Osaka, (S. YASUDA fol. 28 Oct. 1928 HK); Kurose, (Z. TASIRO fol. cum amento ♂ prolept. 13 Jul. 1929 HK); prope Kanazawa, (T. ITIMURA ♀ fr.-Typus *S. vulpinae* var. *tomentosae* HT<sup>1)</sup>).—Prov. **Noto**: Konosumura, (S. KITAMURA ♀ fr. 13 Maio 1929 HK); Hôdatu, (T. SAWADA st. 28 Maio 1923 HT).—Prov. **Ettyû**: Tairamura, (M. NISIZIMA n. 12 ♀ fl. 27 Maio 1934); Ôgayamura, (M. NISIZIMA n. 6 ♀ fr., n. 7 ♀ fl., n. 8 ♂ fl. omnia 10 Maio 1931); Hutomiyamamura, (M. NISIZIMA n. 11 fol. 27 Sept. 1933); Unazuki, (G. KOIDZUMI ♂ & ♀ fl. 18 Apr. 1929); Hannyanomura, (G. KOIDZUMI ♂ & ♀ fl. 15 Apr. 1929.—T. OTAYA fol. Aug. 1930 HK); Takanosu, (T. OTAYA ♀ fl. 12 Apr. 1930 HK); Tateyamaonsen—Huzibasi, (Z. TASIRO fol. 1 Sept. 1929 HK).—Prov. **Etigo**: Yukyuzan, prope Nagaoka, (G. KOIDZUMI ♀ fl. 20 Apr. 1929).—Prov. **Hida**: monte Kuraiyama, (Z. TASIRO fol. 14 Sept. 1929 HK); Simosadani, (Z. TASIRO fol. 5 Sept. 1929 HK).—Prov. **Sinano**: prope Usuitôge, (A. KIMURA n. 519 ♀ fl. 11 Maio & fol. 6 Oct. 1926; n. 523 ♂ fl. 11 Maio & fol. 6 Oct. 1926; n. 528 ♂ fl. 11 Maio & fol. 6 Oct. 1926; n. 553 ♀ fl. 9 Maio 1927 & fol. 6 Oct. 1926; n. 555 ♀ fl. 9 Maio 1927 & fol. 6 Oct. 1926; n. 1231 fol.

<sup>1)</sup>HK indicat Herbarium Universitatis Kyotensis et HT Tokyensis. Specimina sine indicatione speciali in herbario auctoris ipsius asservata sunt.

6 Oct. 1926; n. 1233 fol. 6 Oct. 1926); prope Karuizawa, (A. KIMURA n. 2410 ♀ fr. 8 Jun. 1923); Ōgōmura-Maguse, (T. NAKAMURA ♀ fr. 25 Maio 1930); Mizuhomura-Sinden, (T. NAKAMURA fol. 25 Maio 1930); Iizunahara, (T. SAWADA ♂ & ♀ 18 Maio 1922); Togakusimura, (Collector non indicat. n. 1, ♀ fr. 11 Jul. 1884-Typus *S. vulpinae* var. *tomentosae* HT.—Collector non indicat. n. 2, fol. 11 Jul. 1884 HT); circ. Nagano, (S. MATUDA fol. 26 Jul. 1893 HT).—Prov. Kōtuke: prope Usuitōge, (A. KIMURA n. 512 ♂ fl. 11 Maio & fol. 6 Oct. 1926, infect.; n. 513 ♀ fl. 11 Maio & fol. 6 Oct. 1926, infect.; n. 514 ♀ fl. 11 Maio 1926, 9 Maio 1927 & fol. 6 Oct. 1926, infect.; n. 515 fol. 6 Oct. 1926: n. 1344 ♂ fl. 9 Maio 1926); monte Akagi, (Collector non indicat. ♂ fl. 6 Maio 1878-Typus *S. vulpinoidis* HT); monte Hotaka, (G. KOIDZUMI ♀ fr. 12 Jun. 1914 HT); Haraiti, (NAKAZIMA n. 6 fol. 20 Aug. 1927 HK); Hatomatitōge, (K. HISAUTI n. 576 ♀ fr. 21 Jul. 1934); sine loco speciali, (Collector non indicat. n. 57 ♀ fr. 6 Maio 1878-Typus *S. vulpinoidis*, an vera *S. futura*? HT.—Collector non indicat. ♀ fr. 4 Maio 1888 HT).—Prov. Simotuke: monte Ozaku, (T. MOMIYAMA n. 82 ♀ fr. Maio 1933; n. 79 ♂ defl. Maio 1933); Nikko, (S. KUSANO fol. Aug. 1904 HT); Nikko-Zyakko, (T. MOMIYAMA n. 84 ♂ defl. 1 Jun. 1935; n. 85 ♀ fr., n. 86 ♀ fr., n. 87 ♀ fr., n. 88 ♀ fr., n. 89 ♀ fr., n. 90 ♀ fr., n. 91 ♀ fr., omnia 1 Jun. 1935); Nikko-Umagaesi, (T. MAKINO fol. Sept. 1903 HT); Nikko-Ogurayama, (Y. YABE ♀ fr. & fol. 21 Jun. 1903 HT); Siroyamamura, (Z. TASIRO ♂ & ♀ fl. 20 Apr. 1934 HK); pede montis Kurakakeyama, (H. SEKIMOTO n. 2 ♀ fl. & n. 3 ♀ fl. 18 Apr. 1927); monte Nasu, (T. SAITO ♂ fl. 11 Maio 1935).—Prov. Musasi: Okanobori, (K. HISAUTI fol. HT); Honmatida, (K. HISAUTI fol. HT).—Prov. Hitati: Hukurodamura, (TURUMATI n. 7a fol. 10 Sept. 1933; n. 8 ♂ fl. & n. 9 ♂ fl. 15 Apr. 1934); Kawawadamura, (TURUMATI n. 7b fol. Oct. 1933); Midorigaokamura, (TURUMATI n. 16 fol. 25 Sept. 1934); monte Yamizo, (TURUMATI n. 27 fol. 28 Aug. 1934); circ. Hukuhara, (TURUMATI n. 18 fol. 7 Jun. 1931); Nakazatomura, (E. ISIKAWA fol. cum amentis ♂ prolept. 22 Jul. 1930).—Prov. Iwaki: Kosekimura, (N. IMAI n. 1 ♀ fl. 24 Apr. 1934; n. 4 ♀ fl. 24 Apr. 1934; n. 6 ♂ fl. 20 Apr. & fol. 4 Aug. 1933; n. 10 ♀ fl. 18 Apr. 1933; n. 11 ♂ fl. 18 Apr. & fol. 23 Aug. 1933; n. 14 fol. 3 Aug. 1933; n. 15 fol. 30 Aug. 1933; n. 16 ♀ fl. 27 Apr. & fol. 22 Jul. 1933; n. 17 ♀ fl. 23 Apr. 1933.—A. KIMURA n. 872 ♀ fl. 23 Apr. & fol. 7 Sept. 1934; n. 873 ♀ fl. 23 Apr. 1934, 22 Apr. 1935 & fol. 7 Sept. 1934, 21 Oct. 1934, 15 Sept. 1935; n. 876 ♂ fl. 23 Apr. & fol. 7 Sept. 1934; n. 877 ♀ fl. 23 Apr. & fol. 7 Sept. 1934;

n. 885 ♀ fl. 23 Apr. & fol. 7 Sept., 21 Oct. 1931); Sirakawamati, (T. SAITO n. 31 fol. 26 Jun. 1932); Miharumati, (Y. HATTORI n. 3 fol. 3 Aug. 1935; n. 4 fol. 3 Jun. 1935; n. 5 fol. 18 Jun. 1935; n. 9 fol. 23 Oct. 1935; n. 15 fol. 9 Jul. 1935; n. 27 fol. 22 Jul. 1935; n. 28 fol. 22 Jul. 1935; n. 33 fol. 5 Sept. 1935; n. 31 fol. 5 Sept. 1935; n. 42 fol. 23 Oct. 1935); Ōkumamura, (Y. HATTORI n. 35 fol. 30 Aug. 1935); monte Yamizo, (Z. TASIRO fol. 28 Jul. 1930 HK); Kanamedamura, (KURIMORO ♀ fl. 2 Maio 1931 HK); Taira, (G. KOIDZUMI fol. 21 Aug. 1928 HK).—Prov. **Iwasiro**: Sukagawamati, (S. HATTORI fol. 8 Sept. 1926); monte Mikaguradake, (T. SAITO n. 1 fol. 4 Aug. 1930); monte Kasiyama, (T. SAITO n. 11 fol. 2 Aug. 1931); monte Idesan, (T. SAITO n. 19 fol. 17 Aug. 1931); Urabandai, (Y. HATTORI fol. 3 Oct. 1933); Okinazima, (S. HATTORI ♀ fr. 13 Jun. 1926); Numasawamura-Ōkuriyama, (T. SAITO n. 2 fol. 3 Aug. 1930).

var. **psilocarpa** KIMURA var. nov.

A typo ovariis ima basi paucipilosis et ceterum glaberrimis vel e basi fere ad medium usque minutissime parcissimeque puberulis vel raro omnino glaberrimis discrepat. Ceterum ut in typo.

HAB. JAPONIA. *Honsyū*.—Prov. **Iwaki**: Kosekimura, (A. KIMURA n. 884 ♀ fl. [Typus var.] 23 Apr. 1934 in Herb. A. KIMURA, fol. 21 Oct. 1934. —N. IMAI n. 3 ♀ fl. 21 Apr. 1935).—Prov. **Kôtuke**: prope Usuitôge, (A. KIMURA n. 522 ♀ fl. 11 Maio & fol. 6 Oct. 1926).—Prov. **Simotuke**: pede montis Kurakakeyama, (H. SEKIMOTO n. 1 ♀ fl. 18 Apr. 1927).

12) × **Toisochosenia** KIMURA gen. hyb. nov. (Salicaceae-Salicoideae) (Fig. 2 & 3).

= *Chosenia bracteosa* NAKAI × *Toisusu Urbaniana* KIMURA.

Arbores. Gemmae amentiferae foliiferaeque aequiformes uniperulatae. Perula gemmarum ventrali latere marginibus liberis imbricatis. Cataphyllum primum (in ramulis sterilibus fertilibusque) latere ventrali et secundum dorsali dispositum. Folia adulta alterna oblongo-lanceolata vel oblonga, supra non stomatifera. Stipulae bene evolutae. Amenta coetanea pendula anguste cylindrica foliato-pedunculata. Flores glandulis toto nullis vel interdum tantum una dorsali. Bracteolae spatulato-oblongae apice irregulariter undulato-rotundatae parte superiore rubicundae inferiore pallide flavo-virides. Stamina 5, dispositione ut in *Chosenia*, filamentis liberis glabris; antherae luteae ovaes extrorsae. Grana pollinis normalia nec deformia modo vix variabilia, nucleis 2, interdum 3. Planta ♀ ignota.

Est distinguenda a *Chosenia* floribus ♂ pro parte glanduliferis et a

*Toisusu* glandula masculinorum florum si adest semper una dorsali nec ventrali.

Typus generis hybridi: *Salix kamikotica* KIMURA.



Fig. 2. *Toisochosenia kamikotica* KIMURA. Ramulus amentifer.  $\times 0.6$ .

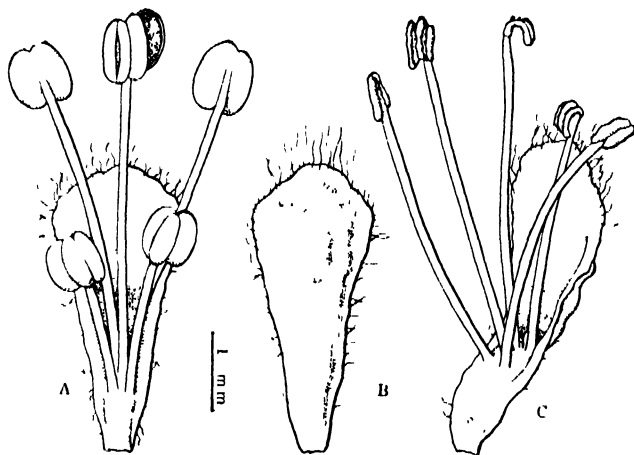


Fig 3. *Toisochosenia kamikotica* KIMURA.

A Flos ♂. B Bracteola a dorso visa.

C Flos ♂ glandulam dorsalem monstrans.

$\times$  *Toisochosenia kamikotica* (KIMURA) KIMURA comb. nov.

Syn. *Salix kamikotica* KIMURA in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol. VI. 2, p. 194 (1931) (Contr. Salic. Jap. IV.).—NEMOTO, Fl. Jap. Suppl. p. 109 (1936) (sphalmat. *kamikotiana*).

NOM. JAP. *Kamikotiyanagi*.

HAB. JAPONIA. *Honsyû*.—Prov. Sinano : Kamikôti, (A. KIMURA n. 782 ♂ [Typus] 16 Maio 1930 in Herb. A. KIMURA ; fl. 31 Maio 1928 ; fl. 1 Jun. 1928 ; gemm. 2 Nov. 1928 ; st. 24 Maio 1929 ; fol. 25 Aug. 1929 ; fl. 11 Maio 1930).

13) *Salix dealbata* ANDERSSON<sup>1)</sup> in Svensk. Vetensk. Akad. Handl. ser. 3, 1850, p. 472 (1851) [Ost-Indiens hittills kända Pilarter (Salices)] ; in Jour. Linn. Soc. IV. p. 43 (1860) ; in Kongl. Svensk. Vetensk. Akad. Handl. VI. p. 8 (1867) (Monogr. Salic.).—SCHNEIDER in SARGENT, Pl. Wilson. III. p. 105 (1916).

? *Salix glaucophylla* ANDERSSON in Svensk. Vetensk. Akad. Handl. ser. 3, 1850, p. 474 (1851) ; in Jour. Linn. Soc. IV. p. 43 (1860) ; in Kongl. Svensk. Vetensk. Akad. Handl. VI. p. 8 (1867) (Monogr. Salic.) (fide SCHNEIDER l. c.).

? *Salix urophylla* LINDLEY ex ANDERSSON in Svensk. Vetensk. Akad. Handl. ser. 3, 1850, p. 487 (1851).—ANDERSSON in Kongl. Svensk. Vetensk. Akad. Handl. VI. p. 5 (1867) (Monogr. Salic.) ; in DE CANDOLLE, Prodr. XVI. 2, p. 194 (1868).—HOOKER f., Fl. Brit. Ind. V. p. 637 (1888) (fide SCHNEIDER l. c.).

? *Salix tetrasperma*,\*\* *urophylla* ANDERSSON in Jour. Linn. Soc. IV. p. 41 (1860) (fide SCHNEIDER l. c.).

? *Salix acmophylla* HOOKER f., Fl. Brit. Ind. V. p. 628 (1888) p. p. (fide SCHNEIDER l. c.).

Descriptioni ab ANDERSSON datae adde : *Perula gemmae ventrali latere libera et imbricata*.

Propter gemmarum naturam primitivam hujus speciei, sectio *Acmophyllae* cujus typus tamen neque adhuc mihi visus est subgeneri *Protitea* KIMURA submittenda esse videtur.

HAB. in India orientali. Examinavi specimina ab U. SINGH ad Dehra Dun anno 1928 lecta.

14) *Salix Dunnii* SCHNEIDER in SARGENT, Pl. Wilson. III. p. 97 (1916).—SHUN-CHING LEE, Forest Botany of China p. 181 (1935).

<sup>1)</sup> Speciem hanc ut sequentes duas obtulit mihi libenter examinandi causa ill. citrologus Dr. T. TANAKA Universitatis Imperialis Taihokensis Professor, cui in hoc loco maximas gratias ago.

? *Salix tetrasperma* (non ROXBURGH) DUNN & TUTCHER in Kew Bull. Misc. Inform. add. ser. X. 255 (1912) (Fl. Kwangtung & Hongk.) (fide SCHNEIDER l. c.).

Descriptioni a cl. SCHNEIDER datae adde: *Perula gemmae ventrali latere marginibus liberis et imbricatis*.

HAB. in Sina australi; Fukien, Kiangsi.—Vidi specimina a H. H. CHUNG in Foochow d. 18 Aprilis 1925 collecta.

15) *Salix dictyoneura* SEEMEN in ENGLER, Bot. Jahrb. XXIX. p. 275, t. II. fig. A-D (1900) (DIELS, Die Flora von Central-China).—SCHNEIDER in SARGENT, Pl. Wilson. III. p. 98 (1916).—HANDEL-MAZZETTI, Symb. Sin. VII. p. 60 (1929).—SHUN-CHING LEE, Forest Botany of China p. 194 (1935).

Ad descriptionem a cl. SEEMEN datam adde: *Perula gemmae ventrali latere marginibus liberis et imbricatis*.

HAB. in Sina centrali et australi.—Vidi specimen a Y. TSIANG ad Tsunyi, prov. Kweichow d. 3 Jun. 1930 collatum.

16) *Salix glandulosa* SEEMEN in ENGLER, Bot. Jahrb. XXI. Beibl. LIII. p. 55 (1896).

var. *Wilsonii* GÖRZ in FEDDE, Rep. Sp. Nov. Reg. Veg. XXXVI. p. 21 (1934).

Syn. *Salix Wilsonii* SEEMEN in ENGLER, Bot. Jahrb. XXXVI. Beibl. LXXXII. p. 28 (1905).—LÉVEILLÉ in Bull. Soc. Bot. France, LVI. p. 301 (1909); in Mem. R. Acad. Ci. Art. Barcelona, ser. 3, XII. no. 22, p. 21 (1916) (Cat. Pl. Kiang-Sou).—SCHNEIDER in SARGENT, Pl. Wilson. III. p. 40 (1916).—REHDER in Jour. Arnold Arb. VIII. p. 93 (1927); ibid. X. p. 112 (1929).—HANDEL-MAZZETTI, Symb. Sin. VII. p. 60 (1929).—W. C. CHENG in Contr. Biolog. Lab. Sci. Soc. China, bot. ser. IX. no. 1, p. 61 (1933) (Enum. Vascular Pl. Chekiang II.).—SHUN-CHING LEE, Forest Botany of China p. 186 (1935).

*Salix Mesnyi* BURKILL in Jour. Linn. Soc. XXVI. p. 530 (1899) p. p. non HANCE.

*Salix Argyi* LÉVEILLÉ in FEDDE, Rep. Sp. Nov. Reg. Veg. X. p. 473 (1912).

*Perula gemmae ventrali latere libera et imbricata ut in typo*.

HAB. Haec varietas imprimis Sinam habitare dicitur. Examinavi specimina a C. Y. CHIAO ad Kulingsze, Nanking, prov. Kiangsu d. 1 Apr. et d. 9 Jun. 1932 lecta.

17); *Salix* subgen. *Protitea* sect. *Nigrae* LOUDON emend. KIMURA.

Syn. *Salix* Cohors I. *Fragiles* KOCH, *Salic. Europ. Comm.* p. 13 (1828) quoad *S. nigram* & *S. Humboldtianam*.

*Salix* sect. *Nigrae* LOUDON [ut Group vii *Nigrae*], *Arb. & Frut. Brit.* III. p. 1529 (1838) pro parte.—SCHNEIDER, *Ill. Handb. Laubh.* I. p. 32 (1904); in *Bot. Gaz.* LXV. p. 5 (1918); in *Jour. Arnold Arb.* I. pp. 2, 5 (1919); *ibid.* III. p. 62 (1921); *Mitteil. Deutsch. Dendrol. Gesells.* XXXV. p. 39 (1925).

*Salix* A. *Amerina* FRIES, I. *Australes* ANDERSSON in *Svensk. Vetensk. Akad. Öfvers.* XV. 3, p. 114 (1858) pro parte.

*Salix* tribus A. *Salices Pleiandrae* a. *Tropicae* stirps IV. *Salices austro-americanae* s. *S. Humboldtianae* ANDERSSON in *Kongl. Svensk. Vetensk. Akad. Handl.* VI. 1, p. 15 (1867) (Monogr. *Salic.*) pro parte.

*Salix* tribus A. *Salices Pleiandrae* b. *Temperatae* stirps V. *Salices amygdalinae* v. *S. triandrae* ANDERSSON in *Kongl. Svensk. Vetensk. Akad. Handl.* VI. 1, p. 19 (1867) (Monogr. *Salic.*) pro parte.

*Salix* A. *Salices Pleiandrae* 1° *Tropicae vel subtropicae* § 4. *Austro-americanae* v. *Humboldtianae* ANDERSSON in DE CANDOLLE, *Prodr.* XVI. 2, p. 199 (1868) pro parte.

*Salix* A. *Salices Pleiandrae* 2° *Temperatae* § 5. *Amygdalinae* ANDERSSON in DE CANDOLLE, *Prodr.* XVI. 2, p. 200 (1868) pro parte.

*Salix* Erste Gruppe *Bitterrindige Baumweiden* K. KOCH, *Dendrologie* II. p. 500 (1872) quoad *S. nigram*.

*Salix* Dritter Stamm *Amygdalinae* DIIPPEL, *Handb. Laubh.* II. p. 223 (1892) quoad *S. nigram*.

*Salix* sekt. *Fragiles* KOEHNE, *Deutsche Dendrologie* p. 89 (1893) quoad *S. nigram*.

*Salix* sekt. I. *Humboldtianae* PAX in ENGLER & PRANTL, *Natürl. Pflanzenfam.* III. 1, p. 36 (1894) quoad *S. Humboldtianam*.

*Salix* sekt. III. *Triandrae* PAX in ENGLER & PRANTL, *Natürl. Pflanzenfam.* III. 1, p. 36 (1894) quoad *S. nigram*.

*Salix* sect. I. *Amygdalinae* BALL in COULTER & NELSON, *New Man. Rocky Mts. Bot.* p. 129 (1909) quoad *S. nigram*.

Arbores, ramulis elongatis, gracilibus et fragilibus. *Gemmae 1-perulatae, perulis ventrali latere liberis et imbricatis.* Folia alterna, utrinque viridia concoloria, lanceolata ad linearia, acuminata, serrulata, vulgo utraque facie stomatibus plus minusve aequinumerosis instructa; petioli eglandulosi vel subeglandulosi. Amenta coaetanea vel serotina, elongato-cylindrica, foliato-pedunculata. Flores ♂ : glandulae 2 ventralis et dorsalis; dorsalis



saepe lobulata. Stamina 3-8, filamentis inferne pubescentibus. Antherae luteae. Flores ♀: glandula una ventralis lata et truncata, basin pedicelli plus minusve semiamplectens, raro dorsalis evoluta. Ovaria glaberrima vel villosa; pedicellis glandulam 2-5plo superantibus, glaberrimis vel pubescentibus; stylis brevibus sed distinctis; stigmatibus brevibus. Bracteolae pallide luteae in ♀ caducae.

Haec sectio propter gemmarum perulas ventrali latere liberas et imbricatas subgeneri *Protitea* KIMURA submittenda est.

HAB. in America a maxime australi per centralem et borealem usque ad prov. Ontario in Canada.

Examinavi *S. nigram* MARSH., *S. Humboldtianam* WILLD. et *S. Goodingii* BALL, quarum plurima specimina aliarumque *Salicum* Pleiandrarum mihi ad examinandum benignissime miserunt Dr. W. A. SETCHELL (Prof. emer. Univ. Californiae), Dr. H. L. MASON (Curator Herb. ejusdem Univ.) et ill. salicologus Dr. C. R. BALL (Washington D. C.). His viris doctissimis gratias quam maximas agere sanctum mihi est officium.

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#### CORRIGENDA

Symb. Iteolog. I in Sci. Rep. Tōhoku Imp. Univ. 4 ser. Biol. X. no. 3.

Pag. 558, lin. 22, loco Tab. I, II., lege Tab. XVIII, XIX.

Pag. 561, lin. infima, loco 2.5, lege 0.25.

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#### EXPLICATIO TABULARUM.

##### TAB. VII.

*Salix Turumatii* KIMURA, Typus.  
Ramuli amentiferi.

##### TAB. VIII.

*Salix Turumatii* KIMURA.  
Ramuli cum foliis adultis.









# CONTRIBUTION TO THE KNOWLEDGE ON THE SOIL MICROFLORA OF *PSEUDOSASA*-ASSOCIATION. III.<sup>1)</sup>

## INOCULATION TEST WITH RHIZOBIA

By

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(With two figures)

(Received July 18, 1936)

*Introduction.* In previous papers<sup>2)</sup>, the author has reported about the distribution of various kinds of microbes, such as filamentous fungi, ordinary pepton-decomposing bacteria, nitrification bacteria, denitrification bacteria, nitrogen-fixing bacteria and so on, in the raw humus soil of the *Pseudosasa*-community on Mt. Hakkoda. As to the presence of Rhizobia there, no experiment actually has been undertaken and it was merely suggested<sup>3)</sup> that the soil may not contain any cells of *Rhizobium* because of the absence of leguminous plants naturally grown there, and also because of the fact that no nodule formation was ever observed on the roots of the leguminous plants which were occasionally raised for some purpose. It is recognized, however, that, so far as acid soils are concerned, the absence of root nodule formation is not always a safe proof of the absence of the root nodule bacteria. It seems to depend on the kind of soil on the one hand, and on the species of plants on the other. Although in most cases, as is recognized in the monograph of FRED, BALDWIN and MCCOY<sup>4)</sup>, the formation of the root nodule and the presence of the agent concerned are observed to take place hand in hand, and the soils of any reaction at which the plant will grow will permit nodule formation in the event of the presence of Rhizobia, nevertheless it must not be overlooked that

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<sup>1)</sup>Contributions from the Mt. Hakkoda Botanical Laboratory, No. 24.

<sup>2)</sup>OKADA, Y. 1931 & 1935. Contribution to the Knowledge on the Soil Microflora of *Pseudosasa*-association. I & II. Sci. Rep., Tôhoku Imp. Univ., Biol., vol. 6, pp. 149-162, vol. 10, pp. 291-298.

<sup>3)</sup>OKADA, Y. 1935. l. c. p. 294.

<sup>4)</sup>FRED, E. B., I. R. BALDWIN & E. MCCOY. 1932. Root Nodule Bacteria and Leguminous Plants. Madison. p. 199.

some investigators noticed<sup>1)</sup>, on the other hand, that in some acid soils, the root nodule formation was not readily observed regardless of artificial inoculation, so long as the soil is not limed. So that it is not absolutely impossible that the root nodule formation is inhibited by some cause or other, even if there are present *Rhizobium* cells. The mere observation in the previous report that the *Phaseolus vulgaris* plants occasionally raised in the Pseudosasetum soil of Hakkoda did not show any sign of root nodule formation may not be sufficient to prove the absence of the bacteria concerned. Further experimental corroboration is needed. For this purpose, two methods may be used. The first is to isolate the *Rhizobium* cell directly from the soil. Considering the present state of the matter, however, this method is extremely difficult or almost impossible. The alternative is first to rectify the soil, e. g., soil reaction, content of mineral matter, etc., in order to favor the root nodule formation, and then to raise leguminous plants in this rectified soil, and later to examine them to see if root nodules are formed or not. As this latter method is far more practicable, the writer attempted some experiments using this principle in the summer of 1935 during his stay on Mt. Hakkoda. A brief note on these experiments is given in the following paragraphs.

**Materials and methods.** The same soil as in the previous report was employed, namely, the raw humus soil of the *Pseudosasa*-community in the compound of the Mt. Hakkoda Botanical Laboratory. It is quite certain that no leguminous plant has ever grown there in this soil. The water content of the soil when sampled was 60%. Its pH-value was 5.2, which was modified, when combined with  $\text{CaCO}_3$ , as is represented in the table below.

TABLE 1.

$\text{CaCO}_3$ (g.) added per 100 g. of fresh soil	0	0.0625	0.125	0.25	0.5	1.00	2.00
pH-value	5.2	6.1	7.1	7.5	7.7	8.2	8.2

pH-value was determined by GILLESPIE's drop ratio method<sup>2)</sup>, with water extract prepared after the direction in ARRHENIUS<sup>3)</sup>. Three kinds

<sup>1)</sup> FELLERS, C. R. 1918. Report on the Examination of Commercial Cultures of Legume-infecting Bacteria. Soil Sci. vol. 6, p. 92.

<sup>2)</sup> SNYDER, E. F. 1935. Methods for determining the Hydrogen-ion Concentration of Soils. U. S. Dep. Agric., Circular no. 56, p. 36.

<sup>3)</sup> ARRHENIUS, O. 1926. Kalkfrage, Bodenreaktion und Pflanzenwachstum. pp. 92-93.

of soils were prepared to raise host plants, namely, untreated soil, soil plus  $\text{CaCO}_3$  (0.7 g.  $\text{CaCO}_3$  per 100 g. of fresh soil, pH-value 7.8-7.9), and soil plus  $\text{CaCO}_3$  and  $\text{K}_2\text{HPO}_4$  (0.7 g.  $\text{CaCO}_3$  and 10 cc. of 3% solution of  $\text{K}_2\text{HPO}_4$  per 100 g. of fresh soil, pH-value 7.8-7.9). These are indicated in the following lines as soils, nos. 1, 2, and 3 respectively. The soils were divided and put into unglazed earthenware pots some 11 cm. wide and 15 cm. deep. About 500 g. of fresh soil were put into each pot. As host plants, three species of Leguminosae were applied, i. e., *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris*. The seeds were first soaked in 0.2%  $\text{HgCl}_2$  solution for about two minutes and then washed thoroughly with sterile water. Four seeds for *Vicia faba*, eight for *Pisum sativum*, and five for *Phaseolus vulgaris* were sown per pot. Half of these pots were inoculated with pure culture of Rhizobia and the remaining half were left non-inoculated. This combination of three soil preparations and two kinds of treatment with respect to inoculation made six sets in all for each kind of host plant, and the experiments were run with duplicate pots for each of these sets. As for the inoculum, pure culture isolated some two weeks previously from the corresponding host plants and kept on yeast-extract mannite agar<sup>1)</sup> as slant culture was applied. In order to prevent contamination, the non-inoculated groups were arranged some 100 m. away from the inoculated groups, with the laboratory building and a patch of bushes between them. The seeds were sown on July 8, 1935. They germinated quite normally in about one week. A few days later, however, the *Phaseolus* seedlings were found seriously damaged by wild hares. Fortunately the *Vicia* and the *Pisum* seedlings were not attacked and they kept on growing. After about 45 days, the plants were all collected, and the number of root nodules and the weight of the tops and the roots were determined.

*Results of the experiment. Vicia faba.* In general appearance, the non-inoculated group were rather slender and of a yellowish green colour, while the inoculated group were more healthy and of a deeper green colour. In height, the two groups were not very different except those in soil no. 1 of the inoculated group, which were lower than any of the others (Fig. 1). None of them flowered in the course of the experiment. Nodules were formed on the roots of all the individuals of the

<sup>1)</sup> FRED, E. B. et al. 1932 l. c. p. 41.





Fig. 1. *Vicia faba*. On the left, the inoculated group, and on the right, the untreated group. The numerals indicate the kind of soil employed.

inoculated group, while there were none on the non-inoculated plants (Fig. 2). The number of nodules increases from soil no. 1 towards soil no. 3. The average dry weight and the number of nodules per plant are given in the table below.

TABLE 2.

	Kind of soil	Dry weight in g.			Number of nodules
		Top	Root	Whole plant	
Non inoculated	no. 1	0.84	1.18	2.02	0
	no. 2	0.87	1.38	2.25	0
	no. 3	0.90	1.65	2.55	0
Inoculated	no. 1	0.95	0.88	1.94	89
	no. 2	1.10	1.49	2.59	111
	no. 3	1.05	1.91	2.95	175

*Pisum sativum*. Both the non-inoculated and the inoculated groups grew quite well, flowered and seeded. A remarkable difference between them was that the inoculated group appeared quite healthy and of a deep green colour, while the other group, although exceeding a little in height, were rather slender and of a yellowish green colour, especially those in soil no. 1. The cause of the superiority of the non-inoculated group in height is yet to be determined. In each separate group, the growth was always best in soil no. 3 and worst in no. 1, soil no. 2 being intermediate. As for the formation of the nodule, the result was almost the same as in *Vicia faba*. All of the inoculated group were positive in nodule formation, and especially profuse formation was noticed in soil no.

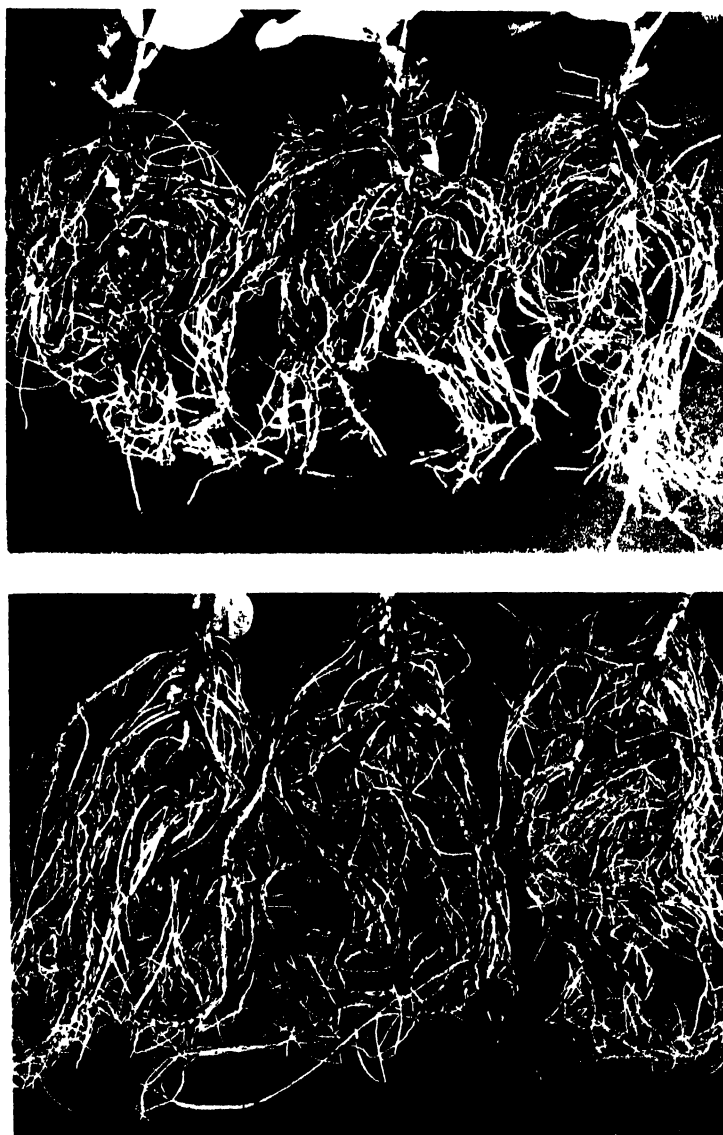


Fig 2 Root of *Vicia faba*, inoculated (above) and non-inoculated (below). Soil no. 1 on the right, no. 2 in the middle and no. 3 on the left.

3. In the non-inoculated group no nodules were formed. The average dry weight and the number of nodules per plant are given in Table 3.

*Phaseolus vulgaris*. As is stated above, the seedlings were attacked

TABLE 3.

	Kind of soil	Dry weight in g.			Number of nodules
		Top	Root	Whole plant	
Non-inoculated	no. 1	0.15	0.28	0.42	0
	no. 2	0.18	0.48	0.67	0
	no. 3	0.15	0.70	0.84	0
Inoculated	no. 1	0.19	0.44	0.63	66 <sup>c</sup>
	no. 2	0.17	0.45	0.62	80 <sup>c</sup>
	no. 3	0.18	0.79	0.97	932 <sup>c</sup>

<sup>a</sup> rather rare on the primary root, development poor in general.

<sup>b</sup> on both the primary and the side roots, development vigorous

by hares shortly after the start and seriously damaged. Although the dry weight was determined with individuals which narrowly escaped the damage, the results were quite irregular, the inoculated group being not always superior to the non-inoculated, which fact may be due to the shortage in the number of the samples. At any rate, the root nodule formation was observed only in the inoculated group, and never in the untreated groups. The average numbers of root nodules per plant (average of 2 to 3 individuals) for the inoculated group were 32, 104 and 283 for soil nos. 1, 2 and 3 respectively.

*Conclusion.* From the results above, it may safely be concluded that *Rhizobium leguminosarum* and *Rh. phaseoli* are normally absent in the raw humus soil of the *Pseudosasa*-community studied here. The same is quite probable for other species of *Rhizobium*. Perhaps the soils of similar plant communities in other localities also may be free from *Rhizobium* cells.

It was shown also that the development of the root nodule is better when the said soil is combined with  $\text{CaCO}_3$  than when untreated, and still better when combined with  $\text{CaCO}_3$  and  $\text{K}_2\text{HPO}_4$ . The cause may be ascribed to the improvement of the general nutrition of the host plants due to the treatments, which fact can be recognized as well with the non-inoculated group.

*Acknowledgment.* The grateful thanks of the writer are due to the Saito Gratitude Foundation by whose help the expense of the present study was partly defrayed.

PRELIMINARY REPORT ON THE BARBELS OF A JAPANESE  
GOATFISH, *UPENEOIDES BENSASI*  
(TEMMINCK & SCHLEGEL)

By

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With three figures

(Received September 12, 1936)

The present goatfish is a common kind among the Mullidae in Japan and lives in muddy bottom near the seashore of the warm sea. This fish possesses the notable character having two long, yellowish, unbranched barbels at the chin (fig. 1). Its length is almost equal to one fifth of the body-length, reaching to the posterior margin of the subopercular bone.

The material used in this investigation was taken from Simoda Bay by dredge-net or "Zibikiami". For histological observations, the barbels were fixed in BOUIN's or ZENKER's solution and then embedded in paraffin, and cut 8-10 micra in thickness. The sections were stained with DELA-FIELD's haematoxylin in combination with eosin, and with MALLORY's triple staining mixture.

I HISTOLOGICAL OBSERVATIONS

The barbel is formed of two layers: the outer is the epidermis and surrounds the inner, which is the dermis (fig. 2).

a) *The epidermis.* Most parts of this layer are composed of oval shaped cells, but a few layers situated above the basal membrane are composed of much elongated cells. Among these epidermal cells, cutaneous taste buds or terminal buds are imbedded numerously, these are homologous with the taste buds on the mucous surfaces of the mouth cavity. The buds of this fish are enormously large in size as compared with those of the other fishes possessing barbels: they are about  $120\ \mu$  in height and  $83\ \mu$  in diameter. They are flask shaped, but are more spherical than those found in the catfish, *Ameiurus* (HERRICK 1901, MAY 1925, OLMSTED 1920); of which apex is not covered by cuticle or hair, but occasionally deepens down the general surface of the epidermis (fig. 3). The bud consists of sensory cells, each being extremely attenuated above the level

of its nucleus which lies near the proximal end of the cells. The sensory cells are arranged regularly side by side, and are situated upon the papillar eminence of the dermis which is raised under the organs. Into this, the nerve fibres enter. Excepting the taste buds, no other organs are found among the epidermal cells.



Fig. 1. Side view of the head, showing two barbels at chin; natural size.

b) *The dermis.* The dermis consists largely of layers of fibrous connective tissue intermingled with muscle cells, blood vessels and xanthophores. But the most parts of the dermis is occupied by a large number of bundles of myelinated nerve fibres. At the middle portion of the dermis, a cylindrical amber-coloured cartilage is

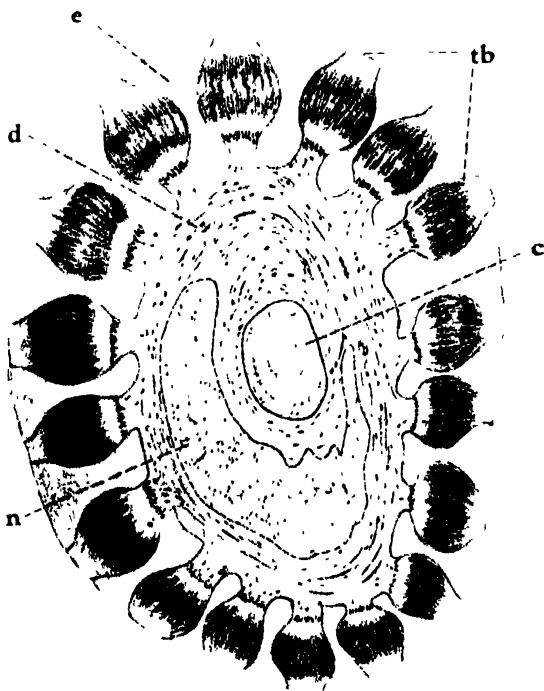


Fig. 2. Cross section of the barbels, showing diagrammatically its structure.  $\times 100$ . c, cartilage; d, dermis; e, epidermis; n, bundles of nerve fibres; tb, cutaneous taste buds.

situated. This cartilage stretches from the base of the barbel to its distal end, forming "supporting rod" of the barbel. The bundles of the nerve fibres above mentioned enclose the ventral half of this cartilage.

## II. EXPERIMENTAL OBSERVATIONS

According to HERRICK (1902, 1903), the cutaneous taste bud has the same function as that of the taste bud on the mucous surface of the mouth cavity. In this sense, the barbel which is supplied with the cutaneous taste buds on its outer surface seems to serve as the organ to recognize food substances. Preliminary examinations were made upon fifteen fishes in the aquarium ( $1.5_m \times 1_m \times 1_m$ ) in order to determine whether this goatfish



Fig. 3. Transection through the cutaneous taste buds which are imbedded among the epidermal cells  $\times 300$

uses these two barbels in the cognition of food substances. These fishes were in nervous condition and refused to eat for about 20 days since being confined in the aquarium. After this period, they took food and seemed to return to normal condition, judging from their behaviours.

This fish is a bottom feeder and remains most of the time quietly at the bottom, but occasionally it swims on the muddy bottom waving the barbels back and forth or left and right. This motion makes a trail on the muddy bottom and seems to be in order to discover food hidden in it, and moreover it is most remarkable when the food substances are given. The goatfish uses the sense of sight in approaching bait, as is judged by dropping a lugworm in the aquarium. This fish did not bite and swallow the worm readily as other fishes having no barbels do, but swallowed it after touching the worm with its barbels. Further, after the lugworms hidden in the muddy bottom, the reaction of the fishes to them was watched. They swam sluggishly on the bottom making trails

with their barbels before detecting and swallowing the hidden food in an average time of two minutes; the goatfishes continued their active trailing on the bottom with their barbels, even after swallowing the food, often returning to the place where the lugworms were found. This experiment was repeated several times with similar results. These results suggest, at first, that the barbels of this fish seem to serve as an organ to recognize and choose food substances.

Next two wads of white cheese cloth of a similar size and appearance, one containing stone and the other some lugworms, were suspended some distance apart in the aquarium. They were observed for fifty minutes, and a record was kept of the number of times each wad was bitten by the fish and the relative positions of the two were changed every ten minutes. At the first time, these two wads were approached by these fishes and touched with their barbels, but the wad containing stone was soon dropped. In a fifty minutes test, the baited wad was bitten more than 40 times, whereas the other was only occasionally bitten. Moreover, four wads of cotton-cloth of similar size but coloured with green, red, black and white, each one containing meat were suspended some distance apart in the aquarium. In this case, too, the four wads were touched with their barbels at first and then bitten by the fishes with nearly equal frequency, having no relation to colours.

When a number of small balls made of American wheat flour were dropped into the aquarium, they were approached by the fishes and touched with their barbels as described already, but were never bitten. Next, these balls were thoroughly soaked in the juice obtained from the lugworms and then dropped into the aquarium. These balls, in strong contrast with the former case, were seized by fishes after touching with their barbels, and bitten, but were quickly vomitted. Further, if the lugworms soaked throughly in the weak solution of acetic acid were thrown into the aquarium, these lugworms were approached by the fishes and touched with their barbels, but they were not bitten. These observations show that the goatfish seems to obtain its food through the use of a chemical sense and its barbels seem to serve as the receptor for this sense.

The part played by the barbels in cognition of food substance can be determined by first eliminating these organs and then testing the fishes. The two barbels were cut at each base and removed from the chin by sharp scissors, without etherization. Twenty-four hours after such an operation, the fishes were fully active, took food, and seemed normal, excepting they swam near the surface of the water more frequently than

the normal fishes did. When the lugworms were dropped into the aquarium in which the operated and normal fishes were, the operated fishes detected more quickly the falling baits by the sense of sight than the normal ones did, and swallowed readily. Next, the reactions of the operated fishes to hidden food in muddy bottom were observed. The normal ones detected readily the hidden food by trailing on the bottom with their barbels as described above, whereas the operated fishes failed to recognize food. This observation was repeated several times with similar results. From this test, the writer believes that the removal of the barbels leads to the loss of the ability to recognize the hidden food.

The lugworms soaked in the weak solution of acetic acid were given to the operated fishes. These worms were swallowed by the fishes, though they were soon disgorged, whereas the normal fishes only touch their barbels at first but take no bait into the mouth as the preceding test shows. If small balls made of American flour were dropped into the water, these too were pounced by the operated fishes and taken into the mouth readily, but soon disgorged, differing from the reaction of the normal ones. These reactions were entirely equal having no relation to whether the balls were soaked in the juice obtained from the worms or not. When two wads of cloth, one with meat hidden in it and the other containing a stone, were suspended in the aquarium, these operated animals nibbled temporarily both packets as though the fish failed completely to distinguish one wad from the other. When, however, these two wads were suspended to an aquarium of the normal fish, the one containing the food was soon surrounded by fishes and nibbled whereas the packet without food was only occasionally nibbled.

From these results, the writer is inclined to favour the view that the eyes and nose in this fish are serviceable in the preliminary steps of procuring food, but whether the material is to be persistently nibbled and finally swallowed depends, as the preceding test shows, on the barbels, and the barbels are necessary to the goatfish in sensing hidden food and choosing the food substances. Moreover, judging from the fact that the present animal lives in a muddy bottom and is a bottom feeder, the barbels seem to be considerably important in its daily activity.

### III. SUMMARY

1. In this paper, the barbels of *Upeneoides bensasi* were observed histologically and experimentally.
2. The barbel is formed of two layers; the outer is the epidermis



and surrounds the inner, which is the dermis.

3. Among the epidermal cells, flask shaped cutaneous taste buds or terminal buds are imbedded numerously. This bud is enormously large in size, as compared with those of the other fishes possessing barbels. The bud consists of sensory cells, which are arranged regularly side by side and is situated upon the papillar eminence of the dermis.

4. The most parts of the dermis are occupied by a large number of the bundles of the myelinated nerve fibres enclosing the ventral half of the cartilage, which is situated at the middle part of the dermis and stretches from the base of the barbel to its distal end.

5. From the preliminary tests, the barbels seem to be necessary to the goat-fish in cognizing the hidden food and choosing its food.

In closing, I should like to express my deep gratitude to Prof. S. HATAI for his kind guidance and encouragement. Further, I should also like to thank Mr. TAKANAGA MITSUI for his kindness in giving me all possible facilities during this investigation.

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# NOTES ON AN EXPERIMENTAL STUDY IN RELATION TO THE EARLY LOCALIZATION OF PRIMORDIAL GERM-CELLS IN THE CHICK EMBRYO

By

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(With Plate IX and twelve text-figures)

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Since the works of SWIFT (1914, '15 and '16) were published the problem in relation to the early localization of the primordial germ-cells in the chick embryo has been studied experimentally by several investigators, by destroying or removing the 'germinal crescent' in various manners. The results of the investigations agreed in confirming SWIFT's view, which seemed to settle the problem. Notwithstanding this, MATSUMOTO put forward a view in 1932, which was fundamentally different from that of SWIFT.

It is not necessary here to make any historical review of the investigations about the primordial germ-cells, because that has already been detailed by MCCOSH (1930) and HEYS (1931) and was also arranged systematically by MATSUMOTO (1932). However, in order to understand the state of opposition existing between the views of SWIFT and MATSUMOTO, their views are here given briefly as follows:

According to SWIFT (1914), the primordial germ-cells arise anterior and antero-lateral to the embryo in a specialized region of the germ-wall endoderm just at the margin of the area pellucida during the primitive streak stage until the embryo has about 3 somites. At first they are carried by their own movement and later by that of the blood. They become to be found in the splanchnic mesodermal tissue near the angle of the coelom in embryos with about 23 to 25 pairs of somites. SWIFT's view is verified experimentally by DANTSCHAKOFF (1931 b and c) and more recently by GOLDSMITH (1935).

According to MATSUMOTO (1932), the primordial germ-cells probably are distributed at the posterior margin of the germ-ring in the stages prior to a 10-hour incubation, and the cells are later found in the posterior portion of the primitive streak including the primitive plate during the

stages of from a 10-hour to a 14-hour incubation. Later, they are transported anteriorly together with the cell-mass of the primitive streak, and reach their position in the anterior portion of the streak. Then, they are given off into the median portion of the lateral mesodermal sheets during the stages of from a 26-hour to a 30-hour incubation. When the lateral mesodermal sheet separates into two layers, the greater number of the primordial germ-cells are arranged in the splanchnic mesoderm, and a smaller number in the somatic mesoderm, during the stages of from a 33-hour to a 50-hour incubation. The multiplication of primordial germ-cells begins even during the stages of from a 10-hour to a 96-hour incubation, but no active migration of primordial germ-cells occurs until the stage of a 96-hour incubation has been reached. MATSUMOTO was, of course, intending to study this problem experimentally also, but owing to unavoidable circumstances, he was obliged to publish his paper as a preliminary report without accomplishing the experimental part.

The earnest purpose of the present work has been to study this problem experimentally. The method tried was cauterization of the 'germinal crescent' and of the posterior portion of embryos. The study was begun in April, 1934, under the kind direction of Prof. Dr. EKITARO NOMURA, to whom the present writer wishes to tender his sincere thanks.

#### MATERIAL AND METHOD

Eggs of white leghorns were used in the present investigation.

In operating, an egg taken from an incubator was put, in order to prevent its being chilled, into a hot water bath kept at 38°-39°C., in which the lower half of the egg was steeped. A fenestra of from 8 to 10 mm. in diameter was opened in the shell, and then an area of the blastoderm or of the embryonic portion was cauterized with a loop of glass capillary which was sheathed around a fine nichrome wire and heated by

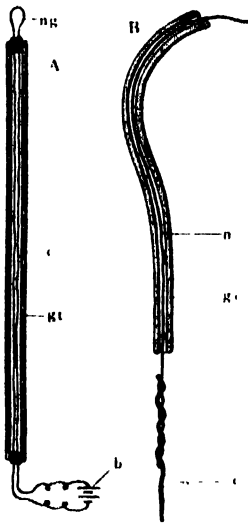


Fig. 1. Cauterization apparatus designed by the present writer. A aspect of the whole apparatus.  $\times 1/2$ . B portion of glass capillary, magnified. b battery, c copper wire, gc glass capillary, gt glass tube, n nichrome wire, ng loop of glass capillary sheathing nichrome wire.

an electric current (Fig. 1). This glass capillary was efficacious in saving the blastoderm from a tear, which without this protection might happen very often, being caused by adherence of the cauterized substance to metal such as a platinum wire. After cauterization, in order to eject air bubbles remaining within the shell, a quantity of the white of another egg, which was kept previously at 38°-39°C., was dropped into the egg, till the concaved surface of the egg substance became convex and came out of the fenestra. A piece of thin mica plate was then put over the fenestra and after the excess of the added white of egg was wiped away, the egg was returned to the incubator. After a half hour or an hour, when the white of egg at the margin of the mica plate dried up, the fenestra was closed by sealing the mica piece with paraffin.

About 250 eggs were operated on, but out of them only 15 survived. For every operation, two non-cauterized eggs were reserved as controls. One of the controls was kept intact and the other opened with a fenestra but soon closed without cauterization.

#### EXPERIMENTAL RESULTS

The cauterization was executed mainly for embryos of incubation of 20 hours or a little more. The cauterization results of the 'germinal

TABLE 1.

Group	Specimen	Hours of incubation before operation	Hours of incubation before fixation	Fixative	Staining	Number of primordial germ-cells found	
						in operated specimen	in non-operated embryo with hours of incubation in parentheses (after MATSUMOTO)
A	219	20.0	61.0	Bouin	Iron haem. with acid fuchsin	6	166 (60)
	227	21.5	62.5	Bouin	Iron haem	0(3)	166 (60)
	233	20.5	73.5	Bouin	Iron haem. with acid fuchsin	0	214 (72)
	248	21.0	67.0	Bouin	Iron haem. with acid fuchsin	2	214 (72)
	253	22.5	48.0	Bouin	Iron haem. with acid fuchsin	0	115 (50)
B	98	5.0	75.5	Zenker	Delaf. haem. and eosin	20	214 (72)
	206	23.0	72.0	Zenker	Iron haem.	43	214 (72)
	242	23.0	96.5	Bouin	Iron haem. with acid fuchsin	164	421 (96)

Group	Specimen	Hours of incubation before operation	Hours of incubation before fixation	Fixative	Staining	Number of primordial germ-cells found	
						in operated specimen	in non-operated embryo with hours of incubation in parentheses (after MATSUMOTO)
C	151	11.5	69.5	Zenker	Iron haem	238	214 (72)
	190	26.5	90.0	Zenker	Iron haem	286	299 (84)
	247	21.0	67.0	Bouin	Iron haem.	347	214 (72)
D	171	19.5	89.5	5% Trichrol.+ 5% Corr. subl	Iron haem.	1(7)	299 (84)
	188	32.0	90.0	Zenker	Iron haem.	13	421 (96)
	250	21.5	48.0	Bouin	Iron haem. with acid fuchsin	0	115 (50)
	251	23.0	48.0	Bouin	Iron haem. with acid fuchsin	0	115 (50)
Non-operated	234		21.0		Iron haem		21 or 18 (24)

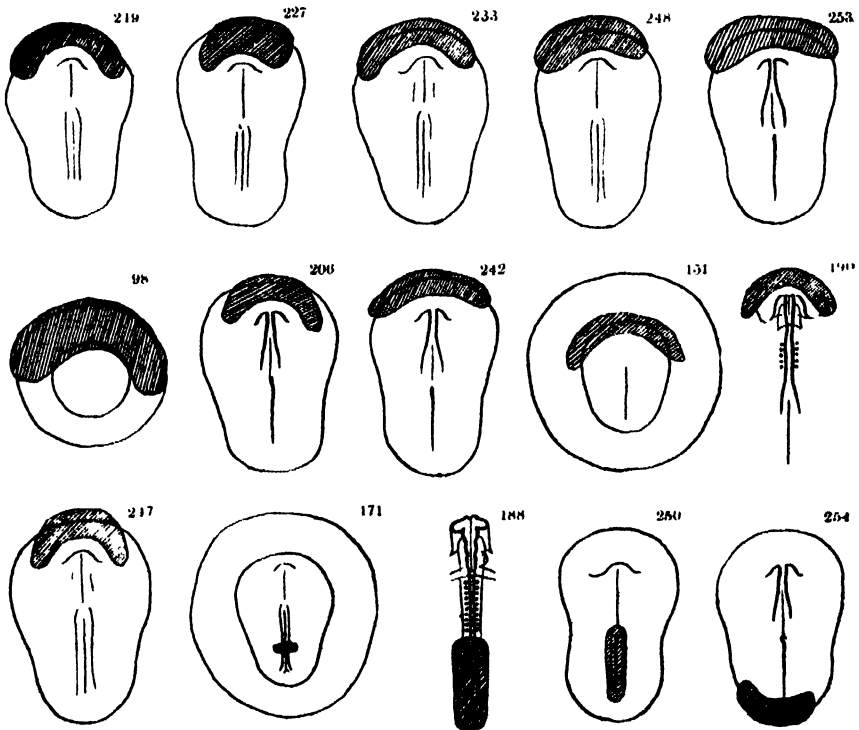


Fig. 2. Sketches of embryos, to show cauterized portion at end of operation. Number given to each sketch in this figure corresponds to that given to specimen in Table 1. Cauterized portion is hatched.

crescent' are grouped in A, B and C, and those of the posterior portion in D, as is shown in Table 1.

### Group A.

The embryos in this group probably received such drastic injury that it was difficult to be certain whether they were living or not. Their hearts, frequently occurring two in one embryo, did not seem to beat, and the vascular system was not well developed in spite of an apparent normality of the blood islands. A microscopical study of the specimens proved a small number of blood corpuscles in the embryonic vessels. The development of embryos was badly delayed. No primordial germ-cells or only a few were found.

*Specimen 219* (Pl. fig. 219). 26 pairs of somites were marked. In sections, 6 primordial germ-cells were recognized in the region of the splanchnic mesoderm: 5 in blood vessels and 1 in the tissue attaching to one of the vessels.

*Specimen 227* (Pl. fig. 227). 26 pairs of somites were counted in this specimen also. 3 cells which approximately resemble the primordial germ-cells were found in the splanchnic mesoderm: 2 in blood vessels (Fig. 3) and 1 in the tissue attaching to one of the vessels.

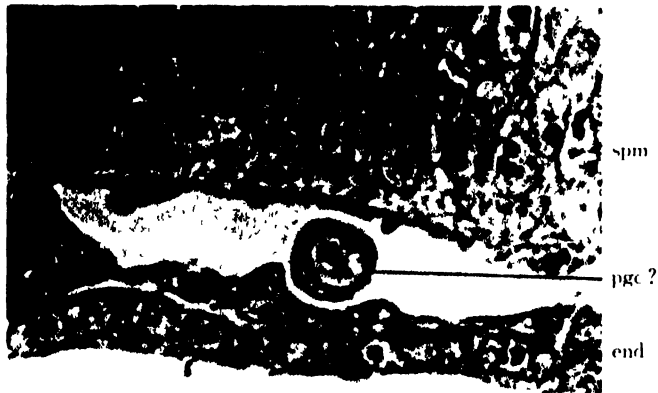


Fig 3 Photomicrograph to show cell which resembles primordial germ-cell in blood vessel.  $\times 1000$  end endoderm, *pgc?* cell resembling primordial germ cell, *spm* splanchnic mesoderm

*Specimen 233* (Pl. fig. 233). Number of somites was 23 pairs. No primordial germ-cells were found.

*Specimen 248* (Pl. fig. 248). 30 pairs of somites were divided. Only 2 primordial germ-cells were contained in the somatic mesoderm near the coelomic angle: one in the coelomic epithelium and the other in the mesenchyme (Fig. 4).

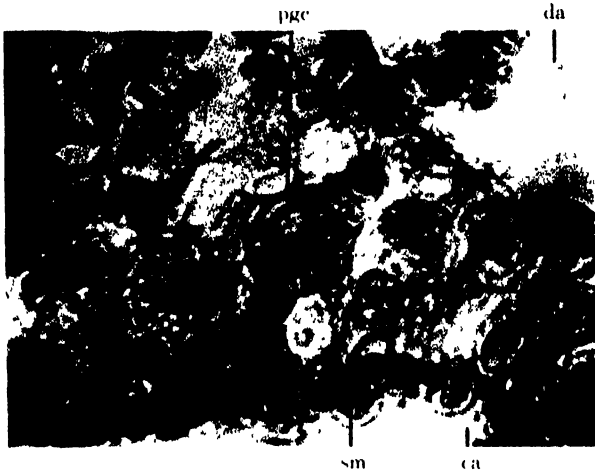


Fig. 4. Photomicrograph to show primordial germ cell in mesenchyme between dorsal aorta and somatic mesoderm  $\times 1000$  *ca* coelomic angle, *da* dorsal aorta, *pgc* primordial germ-cell, *sm* somatic mesoderm

*Specimen 253* (Pl. fig. 253). 23 pairs of somites were counted. No primordial germ-cells were found.

### Group B.

In this group are assembled the specimens in which the number of primordial germ-cells was fewer than that counted by MATSUMOTO in the normal, non-operated embryo. In the specimens, the vascular system was pretty well developed.

*Specimen 98* (Fig. 5). Number of somites was more than 40 pairs. As the anterior portion of the blastoderm was cauterized in an early stage of a 5-hour incubation, it was very doubtful in this case whether or not the cauterized portion corresponded to the 'germinal crescent' in a stage of 20 hours or more incubation. After the operation, 2 holes, one large and one small, appeared on the left side of the embryo. The blood circulation appeared to show some difficulties. 20 primordial germ-cells were counted.

*Specimen 206* (Fig. 6 and Pl. fig. 206). Number of somites was 34 or more pairs. The vascular system seemed to be approximately normal.

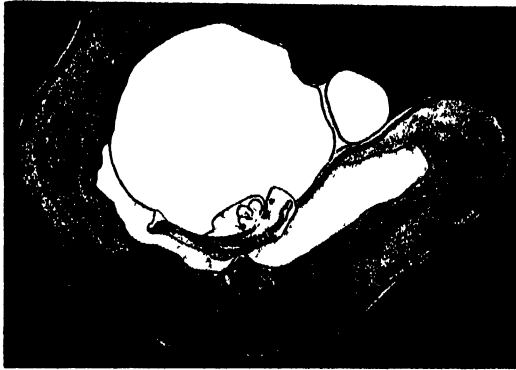


Fig. 5 Sketch illustrating Specimen 98  $\times 3$ .

This embryo, however, had its head twisted towards the left, contrary to a normal case. The primordial germ-cells were 43 in number.

*Specimen 242* (Fig. 7). Neither the extra nor the intra embryonic part showed any apparent abnormalities, but the developmental degree seemed to be somewhat delayed as a whole: probably the cauterization might have been superficial. The primordial germ-cells, when counted, were 164 in number.



Fig. 6 Sketch illustrating Specimen 206  $\times 7$



Fig. 7. Sketch illustrating Specimen 242  $\times 6$  c seam of cauterization



## Group C.

The specimens, which were rich in primordial germ-cells compared with the number counted by MATSUMOTO, are gathered in this group.

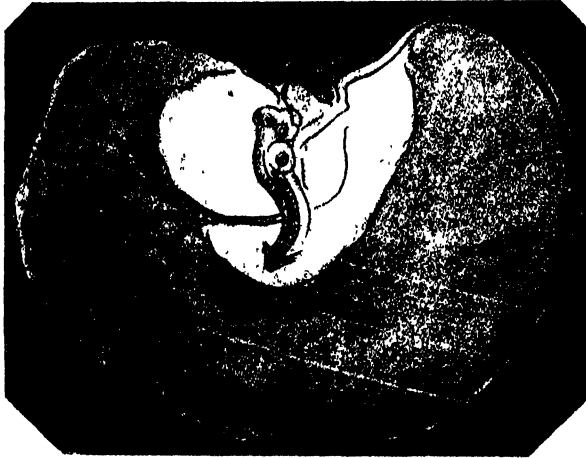


Fig. 8 Sketch illustrating Specimen 151 6

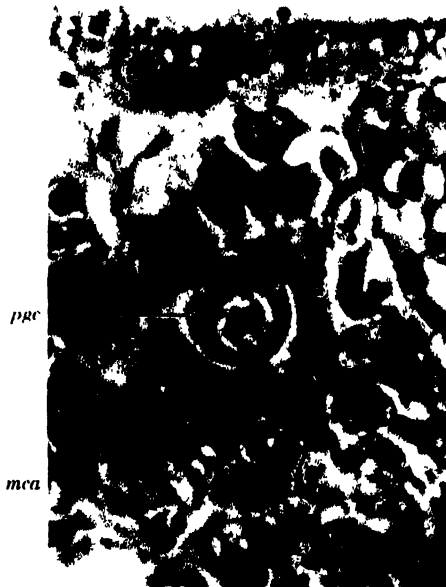


Fig. 9. Photomicrograph to show primordial germ-cell located in mesenchyme near coelomic angle  $\times 1000$ . *mca* mesenchyme near coelomic angle, *prc* primordial germ-cell

Especially, Specimens 151 and 247 contained many cells, even though their blastoderm was considerably injured. All the specimens of this group were very sanguineous.

*Specimen 151* (Fig. 8). The embryo was operated at at the early stage of an 11.5-hour incubation. 238 primordial germ-cells were contained in the mesodermal tissue adjacent to the coelomic angle (Fig. 9)

*Specimen 190* (Pl. fig. 190). This material appeared to be quite normal and may be regarded as most nearly approximating an uninjured specimen among those operated on. The cauterization may have been too slight. However, even though it was slight, a so-called partial castration<sup>1)</sup> must have been made, because the seam of cauterization was distinctly marked on a portion of the 'germinal crescent.'

The number of primordial germ-cells was 286.

*Specimen 247* (Fig. 10). The blood vessels were well developed, even though the portion anterior to the embryo was widely broken. The primordial germ-cells were counted as high as 347.



Fig. 10 Drawing illustrating Specimen 247 . 2.

#### Group D.

The cauterization was executed on the posterior portion of the embryos. Development of the vascular system was very meagre except in Specimen 188.

*Specimen 171* (Pl. fig. 171). The middle portion of the primitive streak was cauterized at the stage of a 19.5-hour incubation. This cauterization may correspond to a partial castration, because according to MATSUMOTO in this stage the primordial germ-cells might be located also in the portion anterior to the cauterization. In many materials at a similar developmental stage, a cauterization of almost the whole primitive streak was practised so that it might be a complete castration. Such a

<sup>1)</sup>The term is used following REAGAN (1916)

grave injury, however, would soon bring embryos to death.

Following the operation, a hole grew in the injured portion and became enlarged to a considerable magnitude. At the end of incubation, the embryo was hanging to the margin of the hole with its body dwindled caudally (compare with Pl. fig. 172). A small amount of blood was contained in the embryonic vessels. Only one primordial germ-cell was found in the mesentery, and in the mesenchyme adjacent to this, 7 cells which resembled the primordial germ-cell were found.

*Specimen 188* (Pl. fig. 188). The embryo of a 32-hour incubation was cauterized caudally from the originating point of the omphalo-mesenteric arteries to the posterior end of the primitive streak. This material developed very vigorously until the fourth night was reached (53 hours after the operation), but the next morning it was found to be dead probably due to some troubles in the vascular system, because a study by sections revealed that the embryonic vessels were filled completely with blood corpuscles. 13 primordial germ-cells were recognized in the mesenchyme near the nephric tissue and in the coelomic epithelium.

*Specimen 250* (Pl. fig. 250). From the anterior portion of the primitive streak to its posterior end was cauterized. A small number of blood cells were found in the embryonic vessels, but no primordial germ-cells were recognized.

*Specimen 254* (Pl. fig. 254). The cauterization was made on the posterior margin of the area pellucida including the posterior portion of the primitive streak. A considerable number of blood cells were found in the embryonic vessels, but no primordial germ-cells were recognizable, in spite of the cauterization being carried on so carefully that the middle portion of the primitive streak remained intact. According to MATSUMOTO this was because some primordial germ-cells ought to be contained also in this portion.

The life and death of both Specimens 250 and 254, similar to those of group A, were indeterminable, and the pulse of their hearts could not be affirmed.

#### Control.

No differences between the intact and fenestrated control eggs were recognized except a slight delay in the development of a few fenestrated ones. Moreover, neither differences between, nor abnormalities of, the primordial germ-cells in either kind of controls were detected.

*Specimen 234* (Fig. 11). In the 'germinal crescent' of this intact material, without considering the attraction spheres, the present writer was able to find some cells containing much quantity of yolk (Fig. 12), very closely resembling those drawn by SWIFT as the primordial germ-cells<sup>1)</sup>, but he was unable to find such cells in the region of the primitive streak mentioned by MATSUMOTO<sup>2)</sup>.

Since the number of primordial germ-cells in each control specimen has not yet been counted, the present writer was compelled to refer to the number counted by MATSUMOTO.

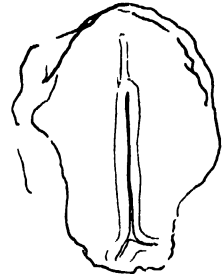


Fig 11 Sketch illustrating Specimen 234 <17

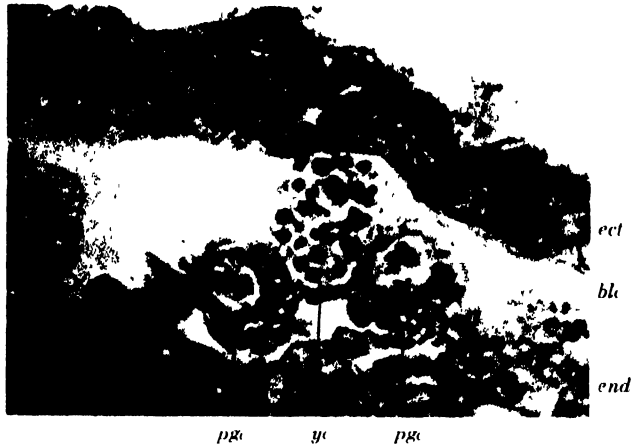


Fig. 12. Photomicrograph to show two cells resembling primordial germ-cells in 'germinal crescent.' 1000. *ble* blastocoel, *ect* ectoderm, *end* endoderm, *pge* cell resembling primordial germ-cell of SWIFT, *ye* mass of yolk granules

### DISCUSSION

In this research of primordial germ-cells, the present writer deemed the round or oval cells of about 15-18  $\mu$  in diameter, each of them containing eccentrically a large, spherical and vesicular nucleus, but the presence of an attraction sphere and of mitochondria was neglected. Accordingly, some questions may arise here in connection with the identifi-

<sup>1)</sup> cf. Fig. 14 of SWIFT (1914).

<sup>2)</sup> cf. Fig. 7 of MATSUMOTO (1932).

cation between what is presumed to be the primordial germ-cells by the present writer and what is so called by other investigators. But the present writer believes that there can not be a great misconception concerning the cells, because cells other than the primordial germ-cells can never exist in the mesodermal tissue near the coelomic angle in the chick embryos of 50-90 hours incubation. For the same reason, the number of primordial germ-cells counted by MATSUMOTO (1932) may be accepted for reference, except those of embryos earlier than a stage of about 25 pairs of somites, which was called by SWIFT (1911) a 'transition stage.'

At the beginning of this investigation, the present writer hoped to verify the 'posterior origin' of primordial germ-cells. In the case of Specimen 231 (21-hour incubation), however, in spite of his careful research in the region of the primitive streak, he could not find any cells to accord with the criteria described by MATSUMOTO. With this single fact, of course, he is not try to deny the advocacy made by MATSUMOTO. The experimental results in Group A and in Specimens 171 and 254 of Group D, however, would not justify MATSUMOTO's view, because in the specimens the entire or larger portion of the primitive streak remained intact and, therefore, the primordial germ-cells ought to be more numerous than those found in the cauterized specimens.

On the other hand, MATSUMOTO's view seems to be confirmed according to two experimental results: one is that the primordial germ-cells were found in Group C, in spite of cauterization of the 'germinal crescent,' and the other is that they could scarcely be found because of the destruction of the posterior portion in Specimens 188 and 250 of Group D. But this proof does not mean the complete support of his view, because of the contradiction of the experimental results in Group A and in Specimens 171 and 254 of Group D.

Then, do these experimental results of the present writer coincide with the experimental proof of DANTCHAKOFF (1931) and of GOLDSMITH (1935) which sustains SWIFT? The result in Group A seems to do so, and also that in Group B can be taken as sustaining SWIFT, if it is considered that the specimens underwent partial castration, notwithstanding the attempt to destroy the whole 'germinal crescent.' Those of the remaining groups C and D, however, are utterly opposed to the experimental proof made by DANTCHAKOFF and by GOLDSMITH. If it were always true that "*apres une destruction partielle du croissant, des cellules germinales sont en effet retrouvées dans les régions gonadiques, mais en nombre moindre*" (DANTCHAKOFF 1934), how shall the specimens in Group C be explained? The

present writer can not give a definite answer to the question as to why so many germ-cells were present in spite of the cauterization of the 'germinal crescent.'

In conclusion, the present writer has no positive data collected giving either negation or confirmation of the views advanced not only by SWIFT, but also by MATSUMOTO.

Finally, from the result obtained in Group C that in all the specimens, which were rich in primordial germ-cells, the vascular system was invariably well developed, it may be considered that the development of primordial germ-cells has some intimate relation with the blood circulation. This consideration appears to be, at least partly, justified by Specimen 188 in Group D. Moreover, if the cauterization on the posterior portion of the embryo were to be taken as a destruction of the anlage of future gonad it may also be considered that the disappearance of primordial germ-cells may depend on the absence of gonad anlage. Therefore, in view of all these considerations, the present writer wishes to advance the proposition that the primordial germ-cells develop abundantly only in the case of the presence of sufficient vascularization and of gonad anlage.

#### SUMMARY AND CONCLUSION

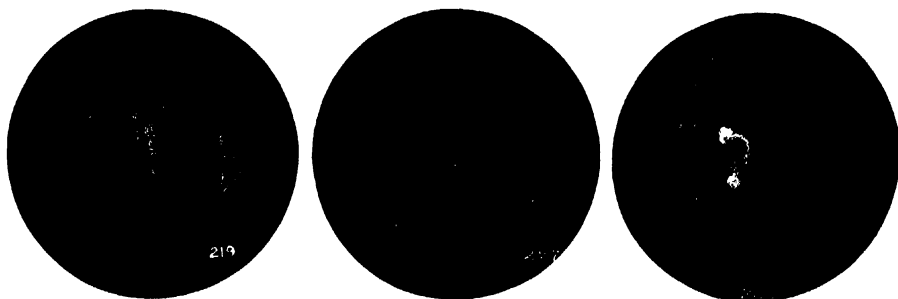
- 1) The present investigation was undertaken in order to test experimentally the theory of 'germinal crescent origin' of the primordial germ-cells in the chick embryo by SWIFT; and that of 'posterior origin' of the same by MATSUMOTO.
- 2) The results of cauterization on the 'germinal crescent' are grouped into A, B and C, and those on the posterior portion into D.
- 3) In Group A, the blood circulation was too feeble, and none or only a few of the primordial germ-cells were contained.
- 4) Group C was enough sanguineous and rich in primordial germ-cells.
- 5) Group B was an intermediate between Groups A and C.
- 6) In Group D, a small number or none at all of primordial germ-cells were found.
- 7) The theories of both SWIFT and MATSUMOTO were indeterminable, because of the dispersed results of the experiments.
- 8) It is suggested that the existence of primordial germ-cells may depend on the presence of a circulatory system and of gonad anlage.

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## EXPLANATION OF PLATE IX

- 171 Photograph illustrating Specimen 171.
- 172 Photograph illustrating Specimen 172 (intact control of Specimen 171).
- 188 Photograph illustrating Specimen 188.
- 190 Photograph illustrating Specimen 190.
- 206 Photograph illustrating Specimen 206.
- 219 Photograph illustrating Specimen 219.
- 227 Photograph illustrating Specimen 227.
- 233 Photograph illustrating Specimen 233.
- 248 Photograph illustrating Specimen 248.
- 250 Photograph illustrating Specimen 250. Ventral view.
- 253 Photograph illustrating Specimen 253.
- 254 Photograph illustrating Specimen 254. Ventral view.



S. HUKAO: Primordial germ-cells in the chick embryo.





# SUSCEPTIBILITY OF PLANARIAN PIECES TO VARIOUS CONCENTRATIONS OF MURRAY-RINGER SOLUTION<sup>1)</sup>

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(With twelve figures)

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During autumn 1932 to spring 1934, experiments were undertaken in which pieces of *Euplanaria dorotocephala* were exposed to various concentrations of Murray's modified Ringer solution. The present paper gives results of a part of these experiments and is concerned with the death frequencies of the pieces. The purpose of the investigation was primarily to determine whether these death frequencies of pieces show an axial differential susceptibility to the solutions as whole animals do to many other agents.

## MATERIAL AND METHOD

Material for the experiments consisted chiefly of head pieces and 1/8 pieces of the postcephalic region of individuals 16 to 18 mm. in body length, but was supplemented by 1/4, 1/6 and 1/12 pieces for certain comparative purposes (Series V). *E. dorotocephala* undergoes fission at a body-level somewhat posterior to the mouth, but without preceding morphological development of the posterior individual. Various lines of evidence have shown however that a physiological demarcation of the region posterior to the fission zone into one or more zooids precedes fission (CHILD, 1910, 1911; CHILD and WATANABE, 1935) and the extreme posterior end is apparently a growing region. In the animals used for experiment the posterior zooid region is well defined physiologically. The worms to be used were subjected to laboratory conditions at least ten days and then starved one week before experimentation. The temperature of the laboratory, under which all the experiments described below were carried on, was adjusted by thermostat to 19-21°C.

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<sup>1)</sup> This investigation was in part supported by the funds from a grant by the Rockefeller foundation in aid of research in the biological sciences at the University of Chicago.

The solution employed here was the modified Ringer solution found by MURRAY (1928) to be most satisfactory for tissue cultures of *E. dorotocephala* and believed to be approximately isotonic. The normal concentration was made up according to the following formula:

NaCl.....	2.5 gm.
CaCl <sub>2</sub> + 2H <sub>2</sub> O .....	0.25
KCl .....	0.05
Distilled water to 1000 cc.	

Concentrations used in experiment are given as percentages of this solution which is regarded as 100 per cent. After making up to a given concentration, the solution was raised to pH 7.6 by addition of a minute quantity of sodium bicarbonate, but no attempt was made to buffer the solution, because any buffer employed, even if not toxic, would introduce effects of the salts contained. The concentrations applied in the present work ranged from zero per cent, i. e., distilled water, up to 300 per cent of the isotonic solution. Exposure time to the solution also varied from 1 1/2 hours to 14 days according to concentration.

The duration of planarian life in distilled water (HESS, 1930) and in diluted Murray-Ringer solution (WATANABE, unpublished data) differs greatly with difference in treatment and is dependent largely on the relative volumes of water or solution and of worms, and on the frequency of renewal of the media during exposure. For this reason, a uniform procedure was followed in all experiments: Pieces were cut in well water and were rinsed three times with the solution to be applied. Then each 20, or 25 pieces from the same body-levels were placed in 200, or 250 cc. of the solution, i. e., 10 cc. of the solution for each piece of animal. After a given exposure period, the solution was poured off and the pieces were rinsed three times with well water, and after 24 hours in well water the number of disintegrated pieces was recorded. The solutions were not renewed at all during exposure.

For the determination of death frequency, the recording of disintegrated pieces is most satisfactory, because disintegration is the most distinct and most decisive evidence of tissue death. In isotonic and hypotonic solutions disintegration occurs immediately after the tissue dies, while in hypertonic solutions it does not appear soon after death, unless the pieces are transferred to isotonic or hypotonic media. Consequently, after exposure to both hypertonic and hypotonic solutions the pieces were always returned to well water for 24 hours before recording deaths.

Since a buffer was not added to the solution and the solution was not changed during the exposure period, more or less change in hydrogen ion concentration, salt content, and other conditions may occur in the solution in consequence of carbon dioxide production, secretion of organic and inorganic substance, etc., by the pieces. Obviously, renewal of the media at such time-intervals as would maintain constant conditions so far as possible would be desirable. However, the rate of change in the solution must differ in different experiments with variation in initial concentration of media and also with difference in body-level from which the pieces were taken. Therefore, in order to keep these conditions constant throughout the period of exposure, the frequency of renewal of media should be different not only for each concentration but for each level of the body. But since determination of the frequency of renewal in each case is practically impossible, solutions were not changed at all in the following experiments.

Since the experimental data concern pieces of equal length from different levels of the body, both length and level of piece are indicated numerically, length as a fraction of postcephalic length, level by a numeral, following the fraction, e. g.,  $1/4-1$ ,  $1/4-2$ ,  $1/4-3$ ,  $1/4-4$ ;  $1/6-1$  to  $1/6-6$ ;  $1/8-1$  to  $1/8-8$ ;  $1/12-1$  to  $1/12-12$ ; etc., the level 1 being the most anterior level immediately posterior to the head which is designated as H in graphs. Results of experiments are presented in a summarized form and are graphed in percentages of dead pieces as ordinates, plotted against levels of the body as abscissae.

#### EXPERIMENTAL RESULTS

*Series I. Effect of 14 day exposure to hypertonic solutions upon death frequencies of  $1/8$  pieces.* Hypertonic solutions in concentration from 140 per cent up to 280 per cent were tested in this series and controls in well water and 100 per cent solution were used. Death frequencies graphed in Figure 1 are averages from 5 lots, each consisting of 20 pieces from each level for each concentration.

During 14 day exposure, no pieces died in well water, 100 per cent, 140 per cent, and 160 per cent solutions, while all pieces died in 260 per cent and 280 per cent solutions. In concentrations from 180 per cent to 240 per cent an axial differential in death frequency appears. As the data in Figure 1 show, the head pieces are most susceptible to the hypertonic solutions. In 180 per cent solution, 80 of 100 head pieces

died, but there were no deaths in pieces from other body-levels (AA, Fig. 1); in 200 per cent solution death frequency of the head pieces is extremely high (98 per cent), as compared with the frequencies in pieces from other levels (BB, Fig. 1). On the contrary, 1/8-pieces from posterior levels are much less susceptible to the solution. In 220 per cent solution (CC, Fig. 1), pieces from the anterior half of the body died in high percentage: 100 per cent in head pieces, 90 per cent in 1/8-1, 85 per cent

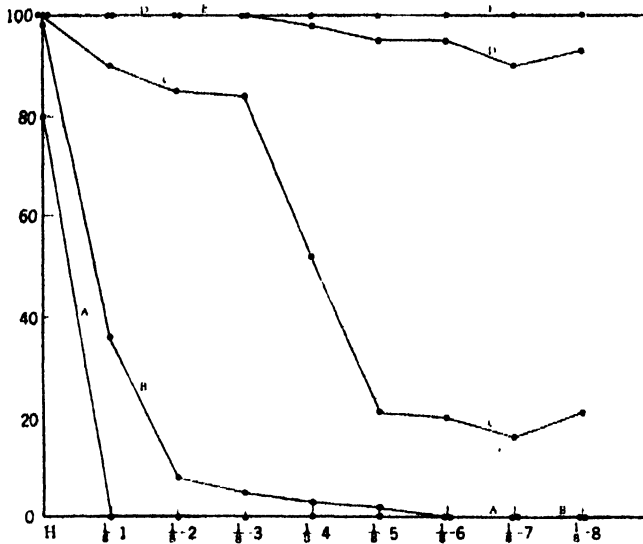


Fig. 1. Death frequencies in heads and 1/8 pieces of postcephalic part of body with 14 day exposure to various concentrations of hypertonic Murray-Ringer solution. Curve AA, 180 per cent; BB, 200 per cent; CC, 220 per cent; DD, 240 per cent; and EE, 260 per cent solution. Abscissae represent level and length of pieces tested, and ordinates death frequency in per cent.

in 1/8-2, 84 per cent in 1/8-3, and 52 per cent in 1/8-4, while pieces from the posterior half died in much lower percentage than those from the anterior half, showing only 16 to 21 per cent. In 240 per cent solution death frequency decreases slightly from anterior to posterior levels with a slight increase in the most posterior pieces 1/8-8 (DD, Fig. 1). This suggests that the region of growth more rapid at the posterior end has a high susceptibility to the solutions as to other toxic agents. Further data concerning this point are given in the following series.

All these data show very clearly that the death frequency in the pieces

increases with the concentration of solution and varies also with body-level in the form of an axial gradient, showing the highest point at the head region.

*Series II. Effect of short time exposures to hypertonic solution.* In this series 250 per cent and 300 per cent solutions were used. To 250 per cent solution the pieces were exposed 18 hours, 35 hours, 48 hours, 72 hours, 96 hours and 108 hours, respectively. The average death frequencies for two lots, each of pieces from 25 worms for each period of exposure are graphed in Figure 2. In the experiment with 300 per cent solution

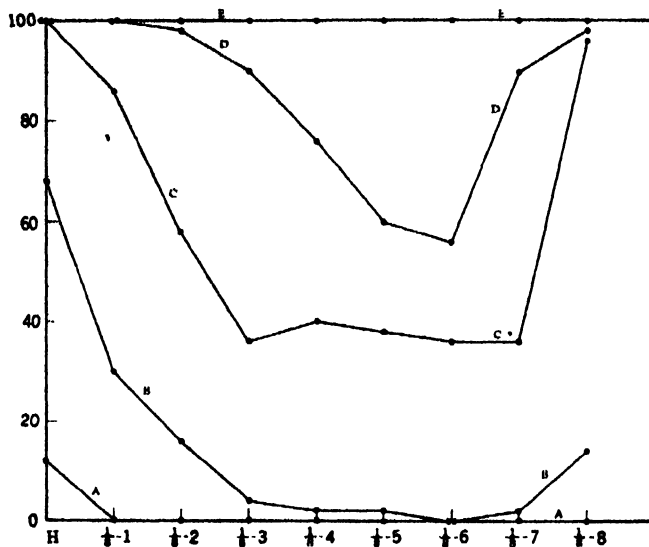


Fig. 2. Death frequencies in heads and 1/8 pieces in 250 per cent solution. Curve AA, 35 hours exposure; BB, 48 hours; CC, 72 hours; DD, 96 hours; EE, 108 hours.

the pieces were exposed 1 1/2 hours, 3 hours, 4 1/2 hours, 6 hours, 9 hours and 12 hours, respectively. Average death frequencies for two lots, each of pieces from 25 worms for each exposure-time are graphed in Figure 3.

In the 250 per cent solution, all pieces were alive 18 hours and all were dead 108 hours after exposure, while in 300 per cent all survived only 1 1/2 hours and all were dead 12 hours after exposure. The differences in complete survival time and complete lethal time in these two solutions indicate the same relation of death frequency to concentration as the preceding series. Between these two limits, death frequency in-

creases with increase in the exposure time for the same concentration. And as Figures 2 and 3 show, for each period of exposure the death frequency is highest in the head region, decreases gradually in the regions

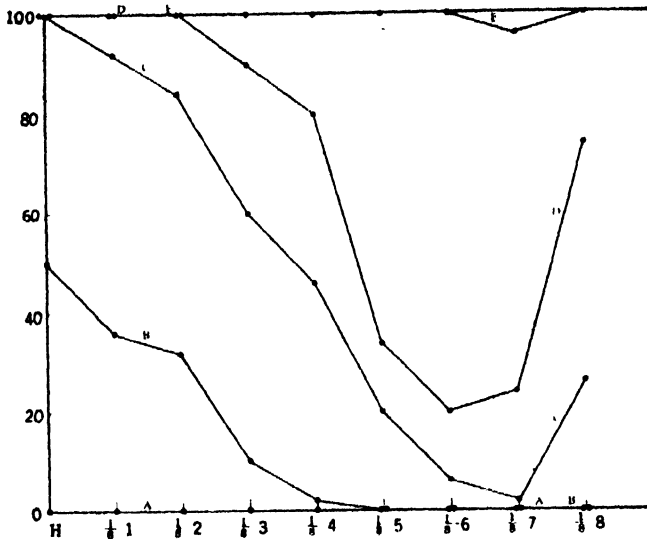


Fig. 3. Death frequencies in heads and 1/8 pieces in 300 per cent solution. Curve AA, 1 1/2 hours exposure; BB, 3 hours; CC, 4 1/2 hours; DD, 6 hours; EE, 9 hours.

posterior to it and increases in the posterior region. These variations in death frequency indicate axial differentials in rate of tissue death in hypertonic solutions.

*Series III. Death frequency in hypertonic solution and physiological conditions of worms.* This series is supplementary to the preceding. As has long been known, in this species the form of the reconstituted head and the frequency of its development, i. e., so-called "head frequency," differ with level of body at which reconstitution occurs as well as length of the piece (see CHILD and WATANABE, 1935, and literature cited there). The head frequency in this species decreases also from the anterior level to the middle, reaching a minimum at the posterior end of the anterior zooid, and increases again at more posterior levels. But its posterior rise is in general greater than that in the death frequency curves in hypertonic solution.

However, so far as the anterior zooid (1/8-1 to 1/8-4) is concerned, head frequency and death frequency run parallel with each other. This

parallelism seems to be rather important. Further evidence for this parallelism is given by pieces of worms collected from Rockford, Ill., in early spring 1934. Head frequencies of these animals were extremely high as compared with those of other material. The upper curve, BB, in Figure 4 shows the head frequency gradient of the Rockford worms

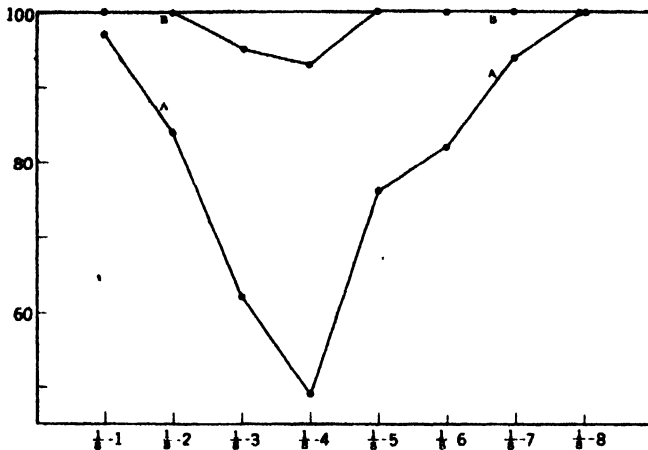


Fig. 4. Head frequency gradients shown by 1/8 pieces of postcephalic part of body. Curve AA, gradient of animals in usual condition; BB, that of animals from a stock showing high death frequency in pieces. Abscissae represent level and length of pieces and ordinates head frequency value calculated by the formula given in the footnote on this page.

and the lower one, AA, that of the worms from the stock used in Series II. Each curve is graphed from the data for 50 worms. The head frequency index was calculated by the method employed in the writer's previous papers (CHILD and WATANABE, 1935; WATANABE, 1935 b<sup>1)</sup>).

The head frequencies of different stocks vary with physiological condition of animals as determined by seasonal changes and other factors in natural environment. These high head frequencies in the worms from Rockford represented a temporary condition, since the head frequencies in worms of other collections from that locality were usually almost the same as those in the material from other localities. 1/8-pieces from this stock were exposed to 250 per cent solution 3 hours, 6 hours, 9 hours, 18 hours, 27 hours, 36 hours and 48 hours, respectively. Average death

<sup>1)</sup>The formula given on page 376, WATANABE, 1935 b,  $(100N_5 + 80N_4 + 60N_3 + 40N_2 + 20N_1 + N_0)/N$  should read  $(100N_5 + 80N_4 + 60N_3 + 40N_2 + 20N_1 + 0N_0)/N$ , as explained in the following paragraph. This is doubtless a misprint.



frequencies for 3 lots of 20 pieces each are graphed in Figure 5 (AA, 3 hours; BB, 6 hours; CC, 9 hours; DD, 18 hours; EE, 27 hours; FF, 36 hours; GG, 48 hours). Comparison of Figure 5 with Figure 2 shows that in Figure 5 death frequency at each body-level is much greater than that at the corresponding level in Figure 2. In the former all pieces were alive only for 3 hours and all were dead 18 hours after exposure,

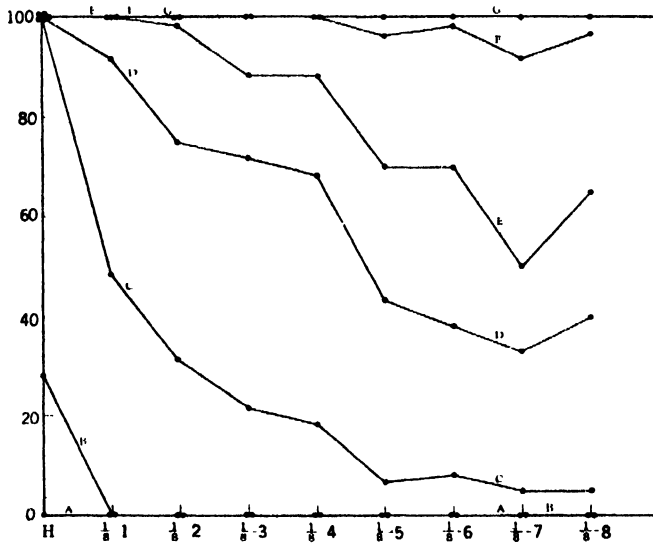


Fig. 5. Death frequencies in heads and 1/8 pieces from animals with high head frequency in 250 per cent solution. Compare with curve BB in Figure 4. Curve AA, 3 hours exposure; BB, 6 hours; CC, 9 hours; DD, 18 hours; EE, 27 hours; FF, 36 hours; GG, 48 hours.

while in the latter all lived 18 hours and all were dead 108 hours after exposure. That is to say, in the Rockford material the complete survival time was one-sixth and the complete lethal time, less than one half of that of the others. These results shown in Figures 2 and 5 and the head frequencies in Figure 4 suggest that the higher the head frequency, the higher the death frequency in hypertonic solution of given concentration. In pieces from posterior zooids, this parallelism does not always appear. These pieces are apparently somewhat more tolerant of the hypertonic solutions, and, particularly in the lower concentrations, do not show increase in death frequency (Compare Figs. 2 and 5 with Fig. 4).

*Series IV. Effect of 14 day exposure to hypotonic solutions on death frequency of 1/8-pieces.* Concentrations used in this series were 1 per

cent, 5 per cent, 10 per cent, 50 per cent, and 80 per cent. Controls in 100 per cent solution and well water showed no deaths. The pieces were exposed to the solutions 14 days, then were returned to well water, and the dead pieces recorded. Average death frequencies for 4 lots each of which consists of pieces from 25 worms for each concentration are graphed in Figure 6.

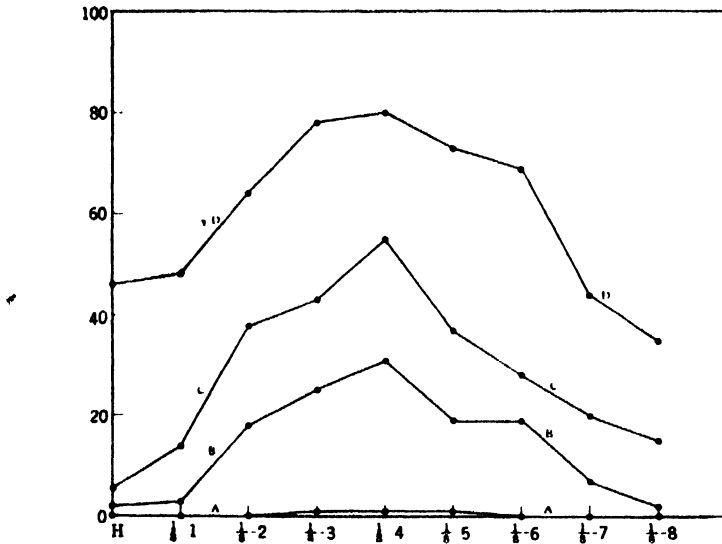


Fig. 6. Death frequencies in head and 1/8 pieces with 14 day exposure to various concentrations of hypotonic Murray-Ringer solution. Curve AA, 50 per cent; BB, 10 per cent; CC, 5 per cent; and DD, 1 per cent.

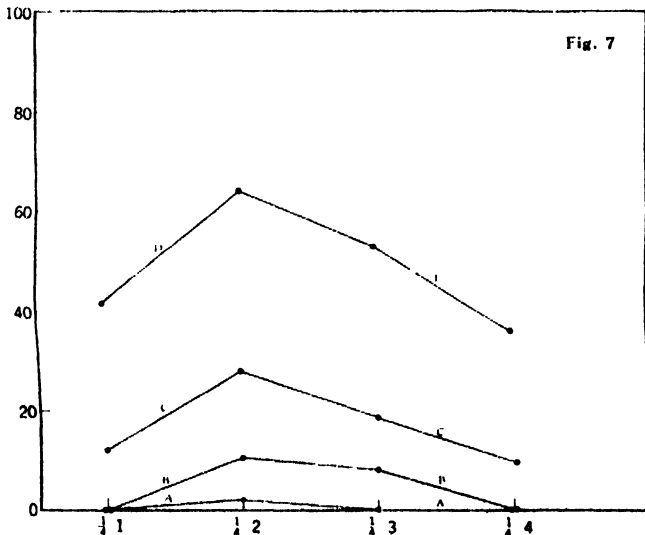
In the course of 14 days, no pieces died in well water, and 100 per cent and 80 per cent solutions. In general, death frequency in pieces increases with decrease in the concentration. But even in 1 per cent solution some pieces still survived after 14 days.

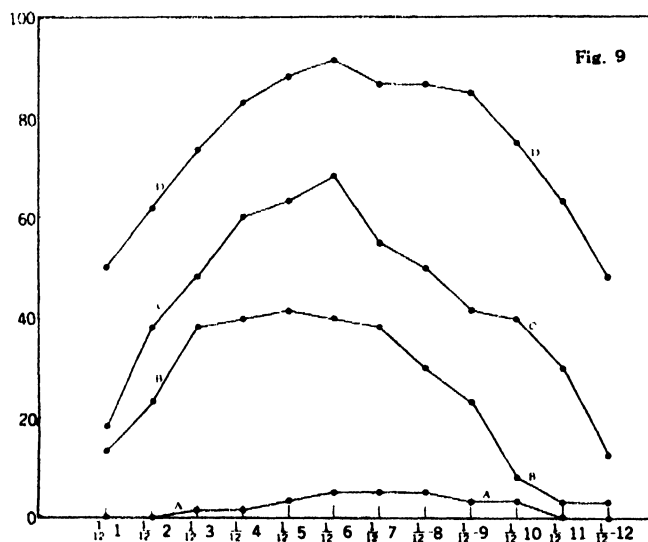
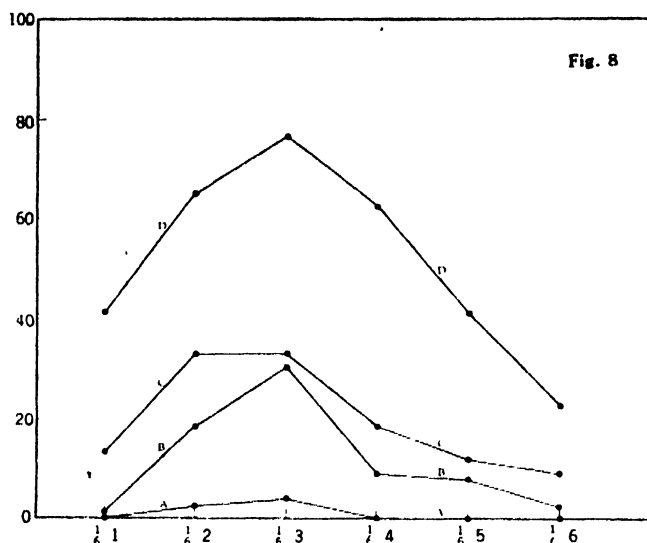
As the graphed data in Figure 6 show, death frequency curves are distinctly different in shape from those obtained with hypertonic solutions. The death frequencies in hypotonic solutions are rather low in the head region, increase to the middle, reaching a maximum at the level of 1/8-4, and decrease posteriorly. So far as the anterior zooid is concerned, in hypotonic solutions the relation of death frequencies to body-level is completely reversed as compared with that in hypertonic concentrations and is also the reverse of the head frequency relation (Compare Figs. 6 and

4). This is of interest as supplementing the data on tolerance or acclimation in the planarian body obtained in earlier experiments (CHILD, 1913, 1914 b, 1916; MACARTHUR, 1920).

*Series V. Length of piece and death frequency in hypotonic solutions.* For the purpose of comparing the death frequencies in pieces of different length,  $1/4$ ,  $1/6$  and  $1/12$  pieces with anterior ends at the same body-level were exposed 11 days to 1 per cent, 5 per cent, 10 per cent, and 50 per cent solutions. Controls in well water and 100 per cent solution showed no deaths. Average death frequencies for 3 lots for each length of piece are graphed in Figures 7, 8 and 9. Each lot of  $1/4$  and  $1/6$  pieces consisted of 25 for each concentration of the solution, each lot of  $1/12$  pieces of 20 for each concentration. In these experiments head pieces were not included.

Figures 7, 8 and 9 show the same relation of death frequency in hypotonic solution to level of body and concentration of solution as has been found in the preceding experiments with  $1/8$ -pieces. It is highest in the posterior region of the anterior zooid, and decreases to both ends of the body, and increases in general with decrease in concentration. Moreover from these data, together with those given in Figure 6 on  $1/8$ -pieces, it may be concluded that for the same concentration, the same period (11 days) of exposure and the same body-level, death frequency increases with decrease in length of pieces. In Table 1, comparative





Figs. 7, 8, 9. Death frequencies in pieces of different lengths with 14 day exposure to various concentrations of hypotonic Murray-Ringer solution. Fig. 7. 1/4 pieces; Fig. 8. 1/6 pieces; Fig. 9. 1/12 pieces. AA, 50 per cent; BB, 10 per cent; CC, 5 per cent; DD, 1 per cent.

data are given for pieces of different length with anterior ends at the same body-level.

TABLE 1.

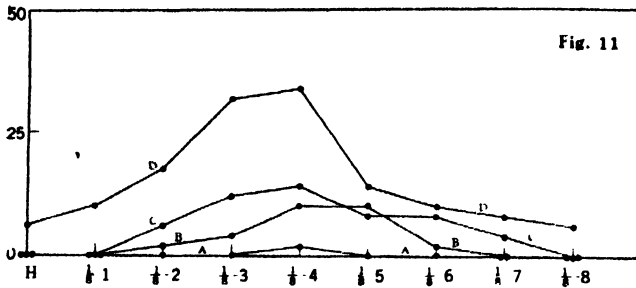
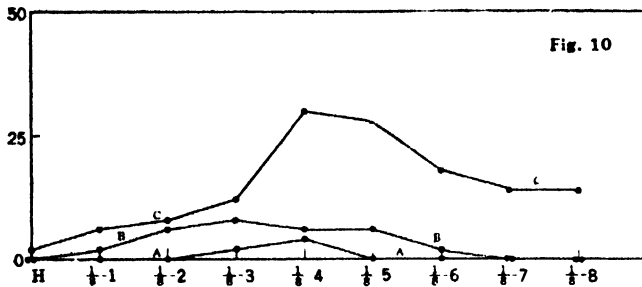
*Death Frequencies of Pieces of Different Length from the Same Body-level in Hypotonic Solutions of Different Concentration. Exposure time: 14 Days.*

Length and level of pieces	Concentration			
	1%	5%	10%	50%
1/4-1	41.0	12.0	0.0	0.0
1/6-1	41.3	13.3	1.3	0.0
1/8-1	46.0	6.0	2.0	0.0
1/12-1	50.0	18.3	13.3	0.0
1/4-2	64.0	28.0	10.6	2.6
1/8-3	78.0	43.0	25.0	1.0
1/12-4	83.3	60.0	40.0	1.6
1/4-3	53.3	18.6	8.0	0.0
1/6-4	62.6	18.6	9.3	0.0
1/8-5	73.9	37.0	19.0	1.0
1/12-7	86.6	55.0	38.3	5.0
1/4-4	36.0	12.0	0.0	0.0
1/8-7	44.0	20.0	7.0	0.0
1/12-10	75.0	40.0	8.3	3.3

*Series VI. Effect of short time exposures of 1/8-pieces to hypotonic solutions.* The concentrations used in this series were 5 per cent and 10 per cent. To 5 per cent solution the pieces were exposed 9 hours, 18 hours and 27 hours, respectively; to 10 per cent solution the pieces were exposed 12 hours, 24 hours, 48 hours and 72 hours, respectively. Average death frequencies for 2 lots of 25 pieces each are graphed in Figure 10 for 5 per cent and Figure 11 for 10 per cent.

Death frequency increases with increase in exposure period, and with each concentration is highest in the middle region (1/8-4), decreasing both anteriorly and posteriorly. Comparison of Figure 10 with Figure 11 shows that death frequency is in general greater in 5 per cent solution than in 10 per cent, i. e., it increases with decrease in concentration.

*Series VII. Effect of exposure of 1/8-pieces to distilled water raised to pH 7.6 by addition of  $\text{NaHCO}_3$ .* As an extreme dilute medium, distilled water was used.  $\text{NaHCO}_3$  was added to give pH 7.6, as in all preceding experiments. The pieces were exposed 3 hours, 6 hours and 12 hours and returned to well water. With 6 hour and 12 hour exposure all pieces died, so that Figure 12 represents average death frequencies for 5 lots



Figs. 10, 11. Death frequencies in heads and 1/8 pieces in 5 and 10 per cent Murray-Ringer solution. Fig. 10, 5 per cent: AA, 9 hours; BB, 18 hours; CC, 27 hours. Fig. 11, 10 per cent: AA, 12 hours; BB, 24 hours; CC, 48 hours; DD, 72 hours.

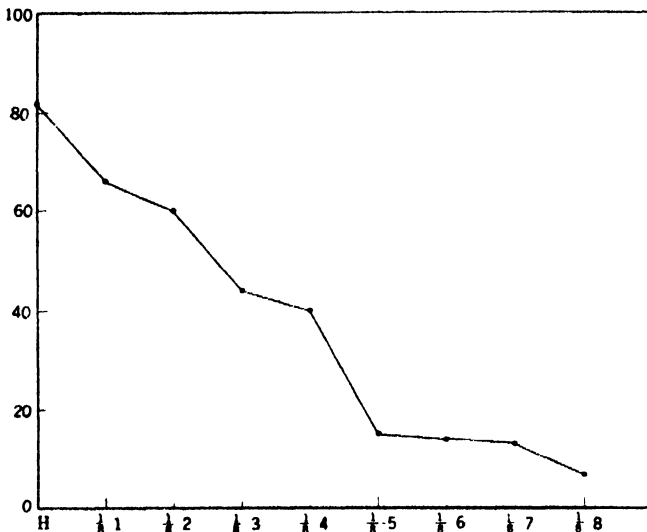


Fig. 12. Death frequencies in heads and 1/8 pieces with 3 hour exposure to distilled water.

with 3 hours exposure of pieces each. As the graph shows, death frequency in distilled water is highest in the head region and gradually decreases posteriorly. The curve is not similar to those of other hypotonic solutions (Figs. 9-10), but resembles more closely those of hypertonic solutions (Figs. 1-3, Fig. 5), except that there is no increase in death frequency in the posterior zooid with distilled water.

#### DISCUSSION

The data of the present paper show, first, that 1/8-pieces of *Euplanaria dorotocephala* live indefinitely a certain range of concentrations (80 to 160 per cent) of Murray-Ringer solution which has been found to be the most satisfactory for tissue culture of this form and is believed to be approximately isotonic (MURRAY, 1928). Second, in concentrations beyond this range, both hypertonic and hypotonic, some pieces die, and death frequency increases in general with increasing concentration above the range of total survival (Figs. 1-3, 5), and also with decreasing concentration below that range (Figs. 6, 10, 11). Third, for a given concentration, it increases with length of exposure period (Figs. 2, 3, 5, 10, 11), and for a given body-level it increases with decrease in length of piece (Figs. 7-9 and Table 1). And, fourth and most important, it shows a definite relation to body-level, from which the piece was taken. In hypertonic solutions death frequency is always highest anteriorly and decreases to the middle region, increasing again at the posterior end (Figs. 1-3, 5), while in hypotonic solutions it is highest in the middle and decreases to both ends (Figs. 6-11). But in distilled water, the frequency is again highest in the head region and decreases posteriorly (Fig. 12).

These definite and orderly variations in death frequency of pieces along the body axis indicate a physiological differential or gradient of susceptibility to lethal action of the solutions. And it is of great interest to note that, so far as pieces from the anterior zooid (H to 1/8-4) are concerned, the death frequency gradients in hypotonic solutions are the reverse of those with hypertonic solutions and with distilled water. A similar reversal occurs in the lethal action of high and low concentrations or intensities of various agents on intact animals. More than twenty years ago CHILD found that in higher concentration of potassium cyanide (m/1000) disintegration begins at the head and proceeds posteriorly in the anterior zooid of this species, while in lower concentrations (0.000025 to 0.000035 molar solution) it occurs much later and usually begins in

the posterior region of the anterior zooid and proceeds anteriorly (Figs. 1-4 and 5-7, CHILD, 1913). A similar difference occurs with different concentrations of ethyl alcohol and various other agents.

The existence of a longitudinal gradient of physiological activity in *Euplanaria* has been established by various lines of evidence, on oxygen consumption (HYMAN, 1923), CO<sub>2</sub> production (ROBBINS and CHILD, 1920), rate of head development (WATANABE, 1935 a) and head frequency (CHILD and WATANABE, 1935). All agree in indicating an activity gradient decreasing from anterior to posterior end of the anterior zooid.

It has been found that in general susceptibility to concentrations or intensities of many agents which are above the range of tolerance and rapidly lethal varies directly with, though not necessary proportionally to physiological activity. Consequently with rapidly lethal concentrations or intensities death begins at the head and progresses posteriorly in the anterior zooid of *E. dorocephala*. With a certain range of lower concentrations or intensities of some agents the ability to tolerate or to acquire tolerance (to acclimate) to the agent decreases from the anterior end posteriorly, consequently with this range of toxic action death begins in the posterior region of the anterior zooid and progresses anteriorly, perhaps over the whole length of the zooid, perhaps only part way, the anterior regions being or becoming completely tolerant and continuing to live indefinitely. The anterior regions which are most susceptible and die first in the one case are most tolerant and die last or not at all in the other. For present purposes the death gradient with rapidly lethal action is called the direct lethal or susceptibility gradient, the gradient with differential tolerance the inverse lethal or susceptibility gradient.

In the lethal hypertonic solutions and in distilled water the death frequency gradient in pieces from successive body-levels of the anterior zooid of *E. dorocephala* is in the direction of the direct death gradient of whole animals (Figs. 1 3, 5, 12): in lethal hypotonic solutions it is in the direction of the inverse gradient of whole animals (Figs. 6-11).

Aside from the question of the cause of death in the hypertonic and hypotonic solutions, it is of interest to note that with decrease in concentration of hypertonic solutions there is no indication of partial differential tolerance in the anterior zooid. In all concentrations in which deaths occur the death frequency gradient is direct. With certain other agents the range of concentration within which the inverse death gradient appears has been found to be rather narrow and it may be possible that further experiment with concentrations intermediate between those near the thresh-



hold of lethality will give evidence of differential tolerance and show the inverse death frequency gradient.

In distilled water the direct death frequency gradient appears, but with increase in concentration of the hypotonic solutions evidence of differential partial tolerance appears and the inverse death frequency gradient results.

In pieces of Japanese planarian called *P. gonocephala*<sup>1)</sup> death frequency gradients similar to those of *E. dorotocephala* appear in Murray-Ringer solution but the ranges of concentration which give direct and inverse death gradients differ somewhat from those reported in this paper (WATANABE, unpublished data). Moreover preliminary experiments indicate that both death frequency gradients can be obtained with different concentrations of LiCl.

It must be pointed out that the death gradient in intact animals is not always the same as the death frequency gradient of pieces from successive body levels with the same concentration. In certain concentrations pieces may die while intact animals may survive and certain other differences have been found (WATANABE, unpublished data). Such differences are doubtless due to the presence of the intact epithelium and body wall in the whole animal and the exposure of internal tissues in the pieces. Also the respiration of pieces is temporarily increased following section and there is some evidence that this change in condition differs in degree in pieces of the same length from different body-levels (CHILD, 1914 a).

As regards death frequency in pieces from the posterior zooid region the relations are less clear. In hypertonic concentrations death frequency decreases posteriorly in this region except for an increase in the most posterior one or two pieces (Figs. 1-3, 5, 1/8-8, 1/8-7). In hypotonic concentrations it decreases from the middle region (1/8-5) to the posterior end. In animals of the length used for experiment the posterior zooid region usually consists of two or three zooids, the most anterior being physiologically determined first the others later in succession. In intact animals susceptibility usually decreases from posterior to anterior zooids

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<sup>1)</sup> Although this species is called *P. gonocephala*, it is so widely different as regards physiological characteristics from the European *P. gonocephala* (see ABELOOS, 1930) that it can not be regarded as the same species. The European species has no posterior zooids and its head frequency decreases distinctly from anterior to posterior end of the body, whereas the Japanese species undergoes fission at a more or less definite level posterior to the mouth (CHILD, 1932), and head frequency is extremely high; even 1/12-pieces from any level of the body develop normal heads in the laboratory water at Sendai.

of this region, and if disintegration does not occur too rapidly the death or disintegration of the different zooids can be distinguished (CHILD, 1913). Evidently the parts of this region constituting different zooids are not in the same physiological condition. Unpublished data on the death gradient of intact animals in hypotonic Murray-Ringer solution are of interest in this connection. Death progresses from the head posteriorly through the posterior zooid region except for a slight increase in susceptibility at the extreme posterior end. This death gradient is very similar to the death frequency gradient of pieces in hypertonic solutions. In corresponding concentrations without calcium death begins at the anterior end of each zooid as well as head of the animal. Similar differences in death in the posterior zooid region have been observed by CHILD (1932) both in *E. dorocephala* and in the Japanese species with KCN in solutions of different hydron concentrations. The facts suggest that the differences are not specific effects of hydron concentration or of presence or absence of calcium, but depend on slight differences in tolerance in different parts of the posterior zooid region.

Data obtained from some tentative experiments by the writer with the different salts of the Murray-Ringer solution indicate that NaCl and KCl increase death frequency and that  $\text{CaCl}_2$  antagonizes the action of these two salts. Results of these experiments as regards action of salts upon death frequency in pieces are completely in accordance with data given by BUCHANAN (1935) on disintegration in this species (see Fig. 2. BUCHANAN, 1935).

#### SUMMARY

1. Death frequencies of pieces of equal length from different body-levels of *Euplanaria dorocephala* are determined in Murray's modified Ringer solution and distilled water, brought to pH 7.6 by addition of sodium bicarbonate.
2. Pieces live indefinitely in 80 to 160 per cent solution of isotonic standard solution. In concentrations above and below this range, some pieces die, and death frequency of pieces from any body-level increases both with increasing and decreasing concentration.
3. In a given concentration of both hypertonic and hypotonic solutions, death frequency increases with increase in exposure period; and for pieces with anterior ends at the same body-level, it increases with decrease in length of pieces examined.

4. Death frequency of pieces also varies with variation in physiological condition of animal as a whole. In hypertonic solutions it is much higher in animals of higher head frequency than those of lower head frequency. This is further evidence of a general relation between physiological condition and susceptibility.

5. In hypertonic solution, death frequency is highest in head pieces and decreases posteriorly, usually increasing again in extreme posterior pieces, while in hypotonic solutions, it is highest in the middle pieces, and decreases to both ends. In distilled water death frequency is highest in head pieces and decreases to the posterior end.

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**FURTHER STUDIES ON THE BARBELS OF A JAPANESE  
GOATFISH, *UPENEOIDES BENSASI*  
(TEMMINCK & SCHLEGEL)**

By

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(Received November 18, 1936)

In the previous paper,<sup>1)</sup> I described the histological structure of the barbels of *Upeneoides bensasi* (Temminck & SCHLEGEL), and concluded, judging from the results of experimental observations, that the eye and nose in this fish serve in the preliminary steps of procuring food, but whether the material is to be persistently nibbled and finally swallowed depends on the barbels, and especially the barbels are necessary to this fish in sensing food materials which are hidden in mud. The present work was carried in the attempt to ascertain more fully the roles played by the barbels in sensing food. In my experiments, I did not think it necessary to make much of the sense of sight because the reactions to hidden food are most important in the case. Accordingly, the problem is then narrowed to the differentiation between the sense of taste, smell and touch in my experiments.

Before going further, I wish to express my hearty thanks to Prof. S. HATAI for his kind guidance in the course of the work, and I will take this opportunity to thank Mr. TAKANAGA MITSUI, in whose laboratory this investigation was carried out.

MATERIAL AND METHOD

The adult fishes used in the present experiment were obtained from Simoda Bay and kept in the aquarium (1 m. by 1 m. by 1.5 m.), as shown in my previous paper.

The operations on olfactory organs and eyes were performed according to the technique worked out by PARKER ('17). The olfactory nerves were cut by incision just posterior to the olfactory pits. In blinding, a pair of eyes were covered with blinders made of thin kid. The blinders were fitted to the surface of fish's head about the eyes and held in position by being stiched to the animal skin at three places. These fishes thus

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<sup>1)</sup> See this number of the Journal, pp. 259-264.

blind folded soon swam about the aquarium as usual, except that they occasionally collided with the walls. For all of the operation the fishes were anaesthetized in 10 per cent solution of ether in sea water.

#### EXPERIMENTAL INVESTIGATIONS

It is questionable whether the barbel is sensitive to both tactile and gustatory stimuli or gustatory alone. To test this point, the first trials were made with a wire which was furnished with a wisp of cotton or a piece of colorless and tasteless gelatin on its hooked end. When this wire was carefully inserted into the aquarium and brought near a fish whose olfactory nerves had been cut, the fish touched it with his barbels but soon ceased to notice the cotton or gelatin. If, however, the cotton was first soaked in the juice obtained from the lugworms and then used in place of the cotton without any treatment or gelatin, the fishes always instantly reacted exactly as to lugworms. No excitation was given to a glass rod.

Having ascertained this difference in reaction to glass rod or cotton without and with treatment by the lugworm juice, the following experiment was done by the use of a fine-pointed pipette, essentially like that of HERRICK ('03 b) and PARKER ('10), directing a jet of water against the fish and then similarly applying a jet of filtered lugworm juice. In the former case the jet was ignored or avoided; in the latter, the reaction was almost similar to that produced by contact with lugworms, as shown already in my previous paper. The evidence from these experiments seems to favor the view that the barbels are more sensitive to gustatory than tactile stimuli, although these gustatory impulses are coordinate with tactile impressions from the same area of skin of barbel.

Next the senses of sight and smell were eliminated by the methods described above, and an attempt was made to ascertain whether this fish is able to recognize his food by aid of his barbel alone. If the lugworms were dropped into the aquarium containing five operated fishes, it was noticed that they were able to detect the lugworms by sweeping the bottom with their barbels after the lugworms fell to the bottom of the aquarium, though they were unable to procure their meat as readily as the normal ones do with use of their sense of sight. The lugworms now being hidden in the mud, the reaction of the fishes to them was observed. They swam trailing their barbels on the bottom and then detected the meats in normal fashion. From these observations, it seems quite clear

that the goatfish obtains its food laying on the bottom by the use of his barbels alone.

At the conclusion of the test, I watched the reactions of the fishes eliminated of their olfactory tracts to two packets of white cheese cloth ; one of these containing lugworms wrapped so as not to be visible and the other made of nothing but cheese cloth were hung in the aquarium, as described in my previous report. These packets were approached by the fishes and then the baited one was sooner or later surrounded and bitten by them, but the packet without meat was soon left from recognition. This test was repeated on the same fishes with essentially similar results. It was quite clear to any one watching these reactions that the fishes notice the difference between the packet of cloth with worms and that without worms, in strong contrast with the fishes eliminated of their barbels. The presence of meat in one of the packets, therefore, may be detected by the fish mainly through the stimulation of the taste buds on the barbels by material emanating from the meat. If so, which receptive organ, smell or taste, is much concerned in the detection of hidden food ? To ascertain this question more fully, I took from among twenty normal fishes two sets of five each and prepared each set differently by subjecting them to special operation. In one set, their peripheral olfactory apparatus was made functionless by cutting their olfactory tracts. From fishes of the other set all the barbels were removed whereby their external gustatory organs were wholly eliminated. At the end of one day, the tests were begun by introducing into the aquarium containing the ten operated fishes a wad of white cheese cloth within which were hidden some lugworms and recording the kind of fish that visited and nibbled it during one hour. These tests were performed on the same fishes for two succeeding days and with similar results as are shown in Table 1.

TABLE 1.

*Number of times at which the packet containing meat was bitten by ten operated fishes during each one hour.*

	Five fishes without olfactory tracts.	Five fishes with their barbels cut.
1	35	20
2	30	16
3	32	14
4	31	15
5	30	12
Mean	32	15

A glance at the above table is sufficient to show that the barbels of this fish are more important in sensing food than the olfactory apparatus.

#### CONSIDERATION AND CONCLUSION

According to BATESON (1890), the majority of fishes seek their food by sight, though there are a few fishes which habitually seek their meat without the help of their eyes. But, there are a number of fishes which use their eyes as the initial steps in the search for food; in other words, they detect the edible substance by other sense from than sight, as for instance the swellfish (COPELAND, '12), the common killifish and catfish (PARKER, '11, '12) and dogfish (SHELDON, '11). All of them secure their food by the use of olfactory sense. Moreover, that the goatfish obtains his food mainly through the external gustatory organ, the barbel, has been shown by my experiments and observations. The epidermis of the barbel of this fish, especially that of anterior edge, as shown already, bears the majority of the cutaneous taste buds without any intervals. The gustatory function by this taste bud was shown by HERRICK ('03 b) and he said: "fishes which possess terminal buds in the outer skin taste by means of these organs and habitually find their food by their means."

This goatfish as well as the other fishes in which the cutaneous taste buds are most highly developed is a bottom feeder. The normal fishes swim trailing their barbels on the bottom and detect the food hidden in the mud in an average of two minutes. This ability to recognize the presence of hidden food was not hindered by the elimination of the senses of smell and sight, but the loss of this ability was caused by the removing the barbels. In addition to this, the possibility to distinguish one packet with meat from the other without any was not influenced by the functionless of the olfactory organ, but this was impossible to the fish whose barbels had been removed. From these results of experiments on the barbels, the barbel may be regarded as a limiting factor in the ability of this fish to recognize hidden food. Now turning to consider the distribution of the taste buds, it is found that there is an intimate correlation between this ability and the distribution of the buds. This fish, differing from the catfish or cyprinoid fishes, limits the distribution of the cutaneous taste buds to his barbels. The elimination of the barbels, therefore, causes the outer gustatory sense to become functionless, and thus this leads the loss of the ability to recognize the presence of hidden food. This correlation is also confirmed by the fact that the fishes without their barbels could not distinguish edible substance from unedible one

until they take these substances into their mouth cavity where the taste buds are numerous.

The question whether the barbel is more sensitive to gustatory or tactile stimuli was also confirmed by my tests. The fish gave no excitation to cotton, gelatin or glass rod, but nibbled quickly at the cotton soaked in the lugworm juice after touching it with his barbels. This reaction was not hindered by the elimination of the olfactory sense. Moreover, the fish without olfactory tracts but with only the normal barbels showed almost the same reactions to a jet of filtered lugworm juice as that produced by contact with the lugworms, but a jet of water was ignored or avoided by this fish. The barbels of this fish, therefore, may be more sensitive to gustatory than tactile. Further, my test showed that the barbel was more valuable in sensing food material than the olfactory organ. In my experiment of one hour, a wad of cheese cloth within which were hidden some lugworms was seized 32 times by fishes without the olfactory tracts but with normal barbels, whereas it was seized 14 times by fishes with their barbels cut. These results of my experiments on the barbels seem to make clear the question.

Here, the question at once arises, does the fish obtain his food mainly by the sweeping the bottom with his barbels even in his natural habitat as that observed in the laboratory? To clear this question, I examined animals found in the mud and then investigated the contents in the digestive canal of this fish. The bottom of Simoda Bay in which this fish was captured is muddy. Upon discovering that the majority of animals contained in the mud obtained from the bottom of this Bay comprised annelidian animals, I was able to ascertain from the microscopical investigations of the fishes at each time they were captured, that the greater parts of the contents in the digestive canal are consisted of the annelidian animals. These results that the contents in the digestive canal definitely coincided with the kind of animal contained abundantly in the mud on which this fish lives, together with the results obtained in my experimental observations, seems to me to prove that this fish detects his food mainly by exploring the muddy bottom with his barbels even in natural habitat as similar as that observed in the aquarium, although he uses the sense of sight in approaching to bait falling down from above.

From the experiments, just summarized, it may be concluded that this fish tastes with the cutaneous taste buds which are embedded among the epidermis of his barbels, essentially as he does with taste buds within the mouth. In short, the barbels of this fish have similar function as that operated by the free ray of pectoral fin of *Trigla corax* (SCHARRER, '35).



## SUMMARY

1. The barbel is more sensitive to gustatory than the tactile stimuli.
2. The ability to recognize the presence of hidden food is not hindered by the elimination of the olfactory sense, but the loss of this ability is caused only when the barbels are removed.
3. The fishes with their olfactory tracts cut but with normal barbels made it possible to distinguish one packet with meat from the other without meat, but the fishes without their barbels made no attempt to distinguish these two packets.
4. The goatfish differs from the catfish or dogfish in detecting his food by the sense of taste rather than the aid of the sense of smell. In other words, this fish tastes and detects his meat with his barbels which bear numerous taste buds on them.

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# A NOTE ON THE DEVELOPMENT OF THE EMBRYO-SAC IN *CARDIOCRINUM CORDATUM*

By

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(With Plate X and five text-figures)

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For a long time cytologists have paid attention to the increase in the chromosome number occurring during the nuclear division in the chalazal region of the embryo-sac in some species of the Liliaceae. The final solution of the problem was, however, found by the result of BAMBACIONI's investigation of *Fritillaria persica*. The results were afterwards confirmed by the same authority and her collaborators in the studies of *Tulipa gesneriana*, *T. praecox*, *Lilium bulbiferum* and *L. candidum*. Recently COOPER has reinvestigated the phenomenon in the case of a number of species of *Lilium*, and has come to the same conclusion.<sup>1)</sup> It seemed to me not to be superfluous to corroborate this important discovery through researches relating to certain species of the Liliaceae, native to our country. So during this summer (1936) *Lilium auratum* LINDL. and *Cardiocrinum cordatum* MAKINO (= *Lilium cordifolium* THUNBERG) were selected for my investigation. The results obtained were about the same in the case of these two plants. For the sake of brevity, a description of the latter species only will be given below.

*Cardiocrinum cordatum* grows abundantly in the suburbs of Sendai. The ovaries of this plant in the different stages of development were collected from the 18th of July to the 5th of August. These were cut transversely in small pieces and fixed in NAVASHIN's solution, after dipping the material in CARNOY's fluid for a few seconds. The sections, 17-25  $\mu$  thick, were stained with NEWTON's gentian-violet-iodine.

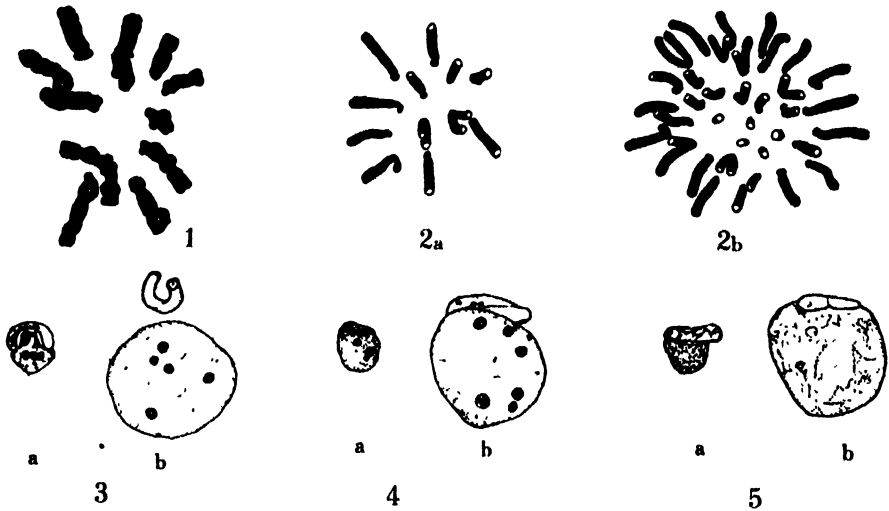
I should here mention that also in this species the embryo-sac mother-cell becomes directly the embryo-sac, the meiotic divisions in the embryo-sac mother-cell proceeding as usual. On the equatorial plate of the first

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<sup>1)</sup> When the manuscript dealing with the present research was completed, ROMANOV's paper on *Gagea* (Planta, Bd. 25, Heft 3, 1936) came into my hands. The writer of this paper has discovered the same phenomenon in the embryo-sac development of this genus.

division, twelve chromosomes were counted (Text-fig. 1).

In the anaphase of this first division, the longitudinal split of each chromosome was clearly visible. After this division, a temporary cell-plate is formed (Pl. X, Fig. 2) which soon disappears. The newly-formed two nuclei separate far apart from each other. There is no recognizable vacuole in the embryo-sac throughout all the stages of development (Pl. X, Fig. 3). The two spindles of the second division are both transverse to the long axis of the embryo-sac (Pl. X, Fig. 4). After the second division, neither cell-wall nor cell-plate is formed, and the four nuclei take random positions in the embryo-sac (Pl. X, Fig. 5). But, in the mean time, three of the four nuclei migrate to the chalazal end, leaving the



Text-figs. 1. Polar view of the equatorial plate of the first division in the embryo-sac. 2. Polar views of the anaphase of the third division in the embryo-sac. a. The one at the micropylar end. b. The one at the chalazal end. 3-5. a. Fusion of sperm nucleus to egg-nucleus. b. Same to secondary embryo-sac nucleus. All  $\times 770$ .

remaining one on the micropylar side. The third division of the embryo-sac development takes place almost simultaneously (Pl. X, Fig. 6). The spiremes are formed in each nucleus and the nuclear membrane disappears (Pl. X, Fig. 7). The spindles of the three chalazal nuclei are distinct for a time, but become later united to form a single one (Pl. X, Figs. 8-10). In the anaphase of this division, the triploid number of chromosomes, that is 36, on the chalazal spindle and the haploid number of the micropylar spindle were counted (Text-fig. 2; a, b). By this division

the second four-nucleate stage of the embryo-sac development is reached (Pl. X, Figs. 11-12). These four nuclei of the embryo-sac divide once more; this is the fourth division of the embryo-sac development. The mitosis of the lower triploid chalazal nucleus is, however, exceedingly anomalous (Pl. X, Figs. 13-14). Three of the four micropylar haploid nuclei form an egg-apparatus and the remaining nucleus migrates to the middle region of the embryo-sac to combine with one of the triploid nuclei, proceeding from the chalazal end (Pl. X, Fig. 15). Thus the complete embryo-sac consists of a three-celled egg-apparatus, a secondary embryo-sac nucleus, and three antipodal cells. The sperm-nucleus which combines with the egg-nucleus is much shorter than the one which combines with the secondary embryo-sac nucleus (Text-figs. 3-5; a, b).

Although the exact morphological features of the chromosomes of this plant could not be examined, two V-shaped chromosomes in the haploid nuclear plate and six of the same shape in the triploid one were clearly observed.

The results of my investigation as above described thus coincide in all main points, with those of BAMBACIONI and COOPER. It seems to me that this type of embryo-sac development is quite common in many species of the Liliaceae.

Grateful acknowledgment is made to Prof. M. TAHARA for much direction in carry on the work.

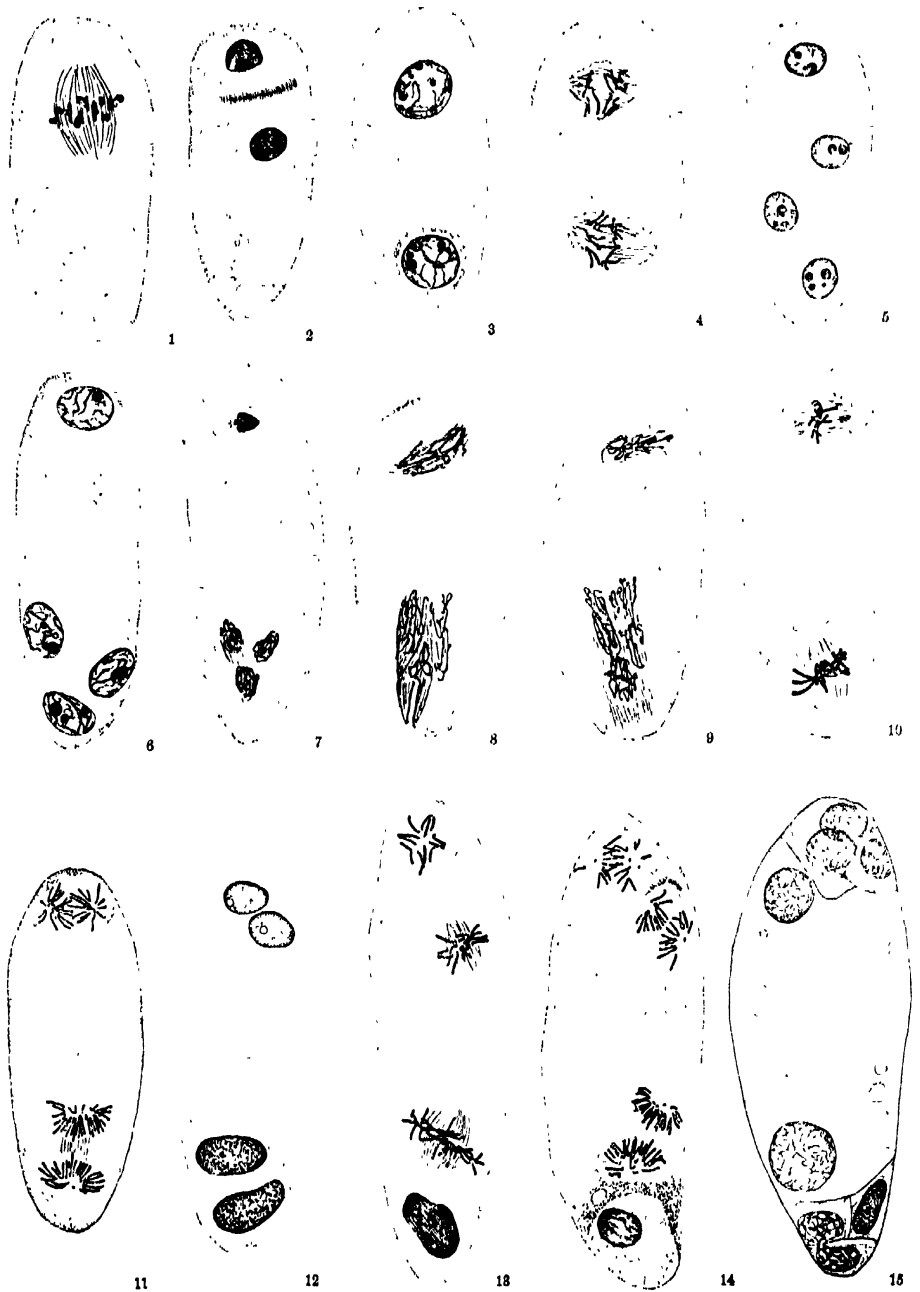
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## EXPLANATION OF PLATE X

All figures are so arranged that the micropylar end of the embryo-sac is towards the top of the plate. Figures are drawn with a magnification of  $620\times$ , and are reduced one-half in reproduction.

- Fig. 1. Embryo-sac mother-cell. Side view of the equatorial plate of the first meiotic division.
- Fig. 2. Side view of the early two-nucleate stage. Cell-plate has been formed.
- Fig. 3. Same, later stage. Cell-plate has disappeared.
- Fig. 4. Side view of the second meiotic division.
- Fig. 5. Early four-nucleate stage.
- Fig. 6. Same, later stage. 3 nuclei have migrated to the chalazal end.
- Fig. 7-11. Third division.
- Fig. 12. Second four-nucleate stage.
- Fig. 13, 14. Fourth division. Mitosis of the basal triploid nucleus is anomalous.
- Fig. 15. Eight-nucleate stage.



K. OIKAWA: Embryo-Sac in *Cardiocrinum*.



# A STATISTICAL INVESTIGATION OF THE CORRELATION BETWEEN CLIMATIC CONDITIONS AND THE EGG-LAYING ACTIVITY OF THE STRAWBERRY WEEVIL, *ANTHONOMUS BISIGNIFER* SCHENKLING<sup>1)</sup>

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(With six figures)

(Received December 19, 1936)

## INTRODUCTION

In previous papers (KATO, 1936 a, b) I have noted a close correlation between the activity of the Strawberry Weevil and meteorological factors such as, the air temperature, the humidity, the duration of sunshine, and so forth.

In this paper I shall deal with the same problems in the light of the results obtained by a further investigation.

## MATERIAL AND METHOD

### 1. Material

The observations were all made at the Gasen-En, a strawberry garden situated on Mt. Dainenji, Sendai, during the period extending from the latter part of April to the middle of June. The field where the experiments were carried on lies nearly in the center of the garden above mentioned and 168 strawberry plants grown there were used (Figs. 1 and 2).

### 2. The Ovipositing Habit of the Strawberry Weevil

In laying its egg the female first makes a hole, piercing the flower-bud through the bract by means of her long snout, and then inserts her ovipositor in the hole and deposits an egg inside of the bud and close to

<sup>1)</sup>The Strawberry Weevil of Japan was formerly called *Anthonomus bisignatus* ROHLFUS by many authors, but more recently the name *Anthonomus bisignifer* SCHENKLING is used (COLEOPTERORUM CATALOGUS, 139, ANTHONOMIDAE, 1934, p. 16).

I am grateful to Dr. HIROMICHI KÔNO for the kind advice given me about the specific name of this weevil.



the pollensac. Afterwards she reaches to the peduncle of the same bud and pierces there again mainly damaging the central cylinder. Each of these flower-buds thus operated on contains as a rule one egg, and either



Fig 1 General view of the strawberry garden on Mt. Dainenji, Sendai.

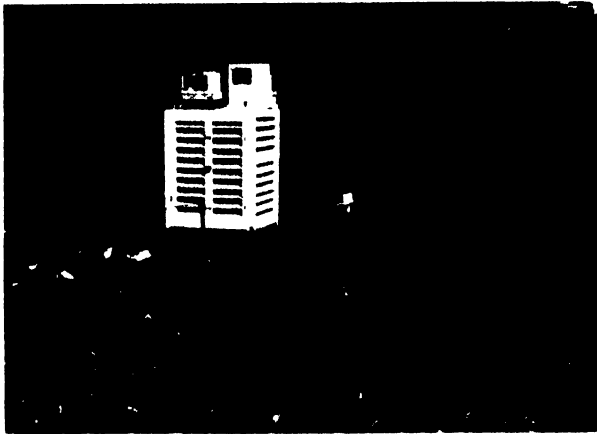


Fig 2. The strawberry garden where the present investigation was made

falls down to the ground or hangs at the tip of the peduncle where it was pierced (KINOSHITA and SHINKAI, 1926).

Consequently if we count the number of injured flower-buds we can know the number of eggs laid by this insect and at the same time we can treat it as an index of the activity of oviposition of the same.

### 3. Observation of the Ecological Climate

The climatic records were cited from those prepared by the Mukaiyama Observatory of the Tôhoku Imperial University in the previous investigations (KATÔ, 1936 a, b).

But the ecological climate must be discussed in such a field-ecology; therefore in the present investigation a meteorological screen was arranged on the experimental field, and the physical condition of the micro-environment was observed,

Since the duration of the sunshine was noticed in the previous observations to be the main factor controlling the egg-laying activity of this insect (KATÔ, 1936 a, b), the solar radiant energy was here chosen as a qualitative factor and thus the total radiant energy was measured.

At the same time I measured in the experimental field the humidity, the air temperature at the level of 30 cm. above the ground and the soil surface temperature. The records of the rainfall were cited from those prepared by the Mukaiyama Observatory.

In measuring the total radiant energy the ROBITSCH-Actinograph was used. It was set about 150 cm. above the ground and was placed on the screen, and the observations were made during the period extending from the second of May to the 31st of the same month. The records of the radiant energy after the first of June were cited from those made by the ROBITSCH-Actinograph which was set on the roof of the building belonging to the Hygienic Institute of the Medical Department of the Tôhoku Imperial University.

The air temperature and the soil surface temperature were recorded during the period extending from the latter part of April to the middle of June by means of OTA's self-recording thermometer set in the experimental field. The humidity was measured by means of RICHARD's self-recording thermo-hygrometer which was set in the screen and placed about 50 cm. above the soil surface.

In recording the evaporation a recording evaporation gauge constructed by myself was used. This evaporation gauge was designed in such a way that the amount of water evaporated from a circular filterpaper of 4 cm. diameter may be measured by means of the fall of a boat floating on the surface of the water contained in a cylindrical tank of 2.55 cm. diameter. The boat above mentioned is connected with a lever and the amount of the loss of water caused by evaporation is thus indicated on the rolling drum by the lever in a scale of 1.85 times as large as the fall of the boat.

### 4. Method of the Statistical Investigation

It is mentioned above that the activity of oviposition of the Strawberry Weevil may be expressed by the number of the injured flower-buds. I examined each strawberry plant and recorded the number of injured flower-buds four times every day; i.e. at the 6th, 10th, 14th and 18th hours. Thus the record prepared at the 6th hour means the number of eggs deposited during the night extending from the 18th hour of the foregoing day to the 6th hour of the next day. And the total records taken at the 10th, 14th and 18th hours express the ovipositing activity of this insect in the daytime. From the records taken at the 10th, 14th and 18th hours we are able to know the number of eggs laid in every four hours in the daytime and also that they show the progression of the ovipositing activity in the daytime.

In the daytime, the air temperature measured at the level of 30 cm. above the ground, the soil surface temperature and the humidity were calculated every hour from the records taken by the self-recording method; therefore the climatic record of every four hours was expressed by the mean values of these five calculated values. In the night the climatic records were calculated every two hours from the records obtained by the self-recording method and are consequently represented by the mean values of seven such calculated values.

It would be rather difficult to show the sudden fluctuation of the total radiant energy unless we calculate it at short intervals from the records taken by the self-recording method. For the reason above mentioned the measurements obtained in such a way as by calculating gr. cal. from the records taken by the self-recording method would therefore be accompanied by much error. In the case of my present investigation the records of the total radiant energy were copied first on COMMERCIAL papers made by the Oriental Photo Industrial Co. Ltd., Japan, and then these papers were cut into three pieces each so as to show a unit of four hours. And thus the total radiant energy of every four hours was expressed by the weight of each of these three and their total weight was recognized as the total radiant energy of the daytime. In this case the error caused by the quality of the copying paper seems to be negligible.

The evaporation was shown in mm. by relative values calculated directly from the records taken by the self-recording method.

The existence of the correlation between the number of eggs laid and the climatic records above alluded to and their degrees were investigated by the method of calculating the coefficients of correlation in the following three series.

i) In the daytime the coefficients of correlation were calculated between the number of eggs laid and the climatic records. From these coefficients thus obtained we can notice the existence of a correlation between the activity of the oviposition and the climate, and are able to know the degree of correlation from the same.

ii) The correlation in the night was discussed in the same way as that mentioned above.

iii) In the daytime the number of injured flower-buds and the climatic factors were measured every four hours, namely at the 10th, 14th and 18th hours. Since in the period extending from the 6th to the 10th hour of each day it is conceivable that the climatic condition and therefore the activity of the oviposition are in a similar phase each day, the coefficients of correlation between the number of eggs laid and the climatic factors of this part of the daytime were calculated. Accordingly the correlation of the other parts of the daytime, namely from the 10th to the 14th hour and from the 14th to the 18th hour, were discussed in the same manner. By these investigations it is clear that the main factor controlling the egg-laying activity changes with the progression of time.

Now we have here the coefficients of correlation in three series, but in the case of the practical calculation of these coefficients the mean curves of the frequency distribution showing the number of eggs laid daily and those of the daily climatic fluctuation were first prepared, and then the deviations between the mean values and the recorded values were calculated in the terms of the percentage of the mean values, and the coefficients of correlation were calculated among these deviations, and lastly SHEPPARD's correction was applied.

In the present statistical investigation the data from the first part of the period of oviposition, namely before the 9th of May, together with that from the latter part of the same, i.e. after the second of June, were neglected, as the number of eggs laid in these two periods was too small to have any statistical meaning.

In the case of rainfall, the correlation was discussed without calculating the coefficient of correlation.

## RESULTS

### 1. The Time and the Diurnal Activity of the Oviposition

In 1936 the oviposition of this weevil was observed for the first time on the 4th of May and it becomes suddenly more active after about the

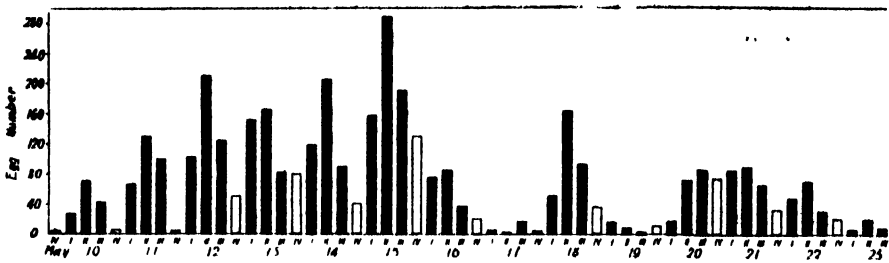


Fig. 3. Frequency histogram showing the number of eggs laid by

10th of the same month being influenced by a good climatic condition. It reached the climax on the 15th of the same month. After the 20th of the same month the activity of egg-laying gradually decreased and after the 11th of June it had nearly ceased, there being found no flower-buds injured by these insects (Fig. 3).

From the diurnal activity of the egg-laying it is learned that the egg-laying activity increases rapidly after sunrise and becomes most active at noon, then it decreases towards the evening, and only a small number of eggs are laid during the night.

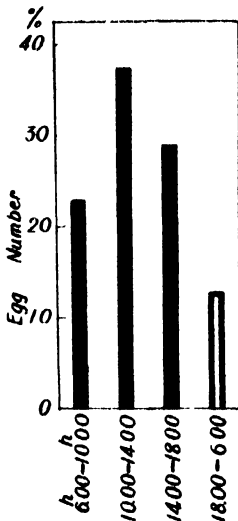
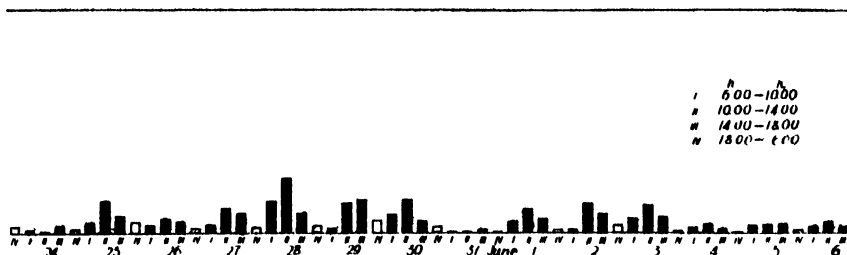


Fig. 4. Frequency histogram showing the diurnal activity of the oviposition of the Strawberry Weevil.

According to the data taken during the period extending from the 10th day of May to the first of June (Fig. 4), the number of eggs laid in the morning is  $22.6 \pm 1.2$  percent of the total number of one day, and  $37.1 \pm 1.7$  percent of the same are laid in the noon-time period from the 10th hour to the 14th hour and in the evening the number decreased to  $28.7 \pm 1.8$  percent.

Therefore 88.4 percent of all the eggs laid daily are deposited in the daytime and only the remaining 11.6 percent are laid in the night. Now it must be noted that the difference between the 22.6 percent of the morning and the 28.7 percent of the evening is not to be considered negligible, as it is just significant statistically.

In conclusion we may say that the oviposition of the Strawberry Weevil takes place mainly in the daytime being most active at



the Strawberry Weevil during the egg-laying season of 1936.

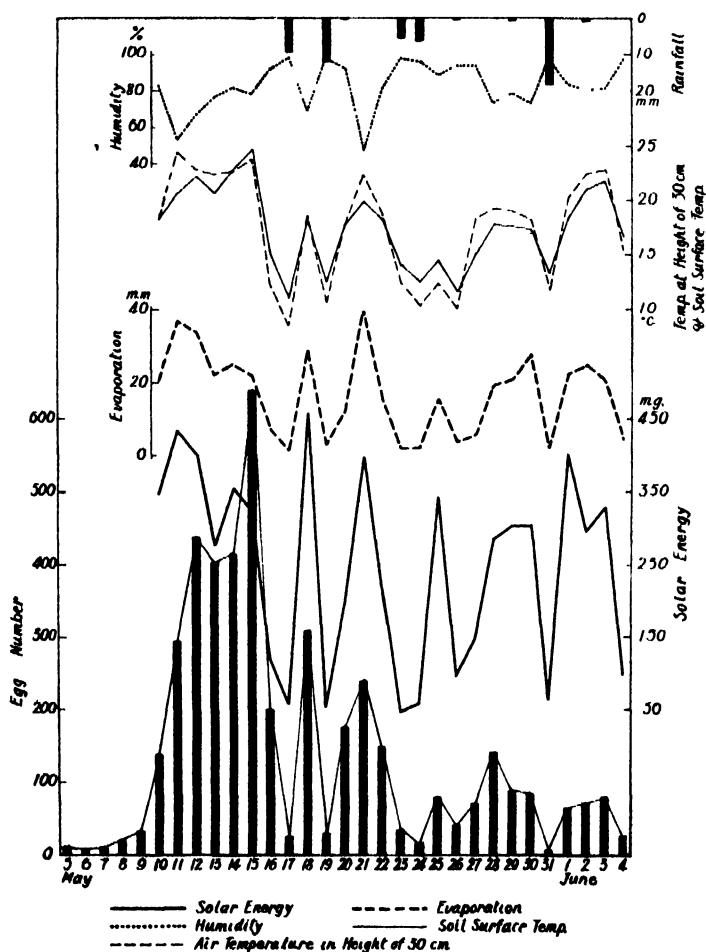


Fig. 5. Correlation figure showing the frequency histogram of the eggs laid in the daytime and the variations of the climatic factors of the same.

about noon, and we may notice the tendency that the afternoon is superior to some extent to the morning in the number of eggs laid, though they stand in a nearly symmetrical relation as against the number of the noon hour.

## 2. Correlation between the Activity of the Oviposition and the Climatic Factors.

From Figures 5 and 6 which show the correlation between the number of eggs laid daily and the daily variation of the climatic condition, we may also find a close correlation between the ovipositing activity and

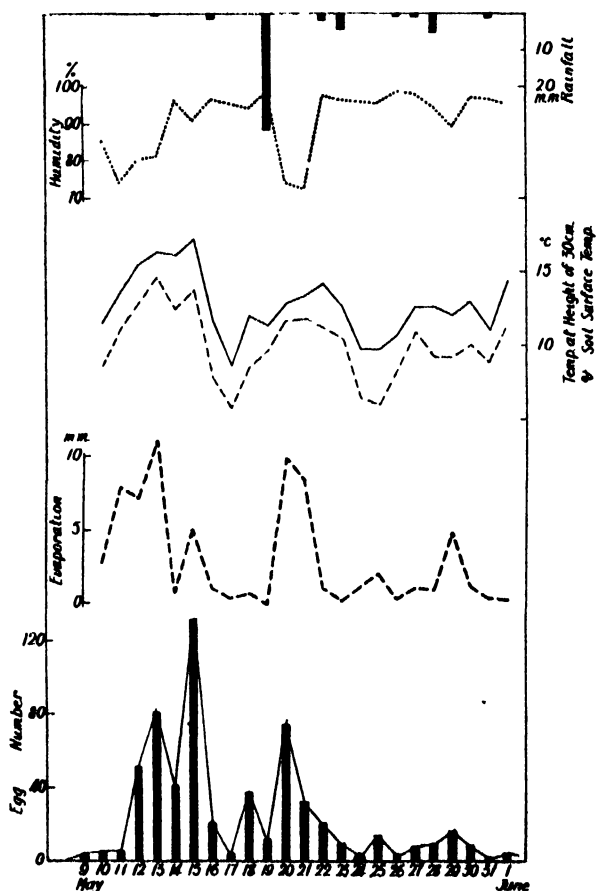


Fig. 6. Correlation figure showing the frequency histogram of the eggs laid in the night and the variations of the climatic factors of the same.

some of the climatic factors. When the number of eggs laid is increased, there is always an accompanying increase of the total radiant energy, of the evaporation, of the air temperature measured at the height of 30 cm. above the soil surface and of the soil surface temperature and lastly there is a decrease of the humidity. Now the degree of these correlations will be discussed in the following pages, calculating the coefficients of correlation (Table 1).

i) As a general rule, the values of the coefficients of the correlation of the total radiant energy and the soil

TABLE 1.

*Coefficients of correlation calculated between the climatic factors and the activity of the oviposition of the Strawberry Weevil.*

Time-Interval h — h	Total Radiant Energy	Soil Surface Temperature	Air Temperature	Evaporation	Humidity
6.00-10.00	$0.884 \pm 0.032$	$0.645 \pm 0.084$	$0.822 \pm 0.047$	$0.928 \pm 0.020$	$-0.631 \pm 0.087$
10.00-14.00	$0.813 \pm 0.023$	$0.932 \pm 0.018$	$0.915 \pm 0.023$	$0.921 \pm 0.023$	$-0.766 \pm 0.058$
14.00-18.00	$0.740 \pm 0.064$	$0.839 \pm 0.042$	$0.825 \pm 0.045$	$0.719 \pm 0.068$	$-0.561 \pm 0.096$
6.00-18.00	$0.906 \pm 0.025$	$0.834 \pm 0.018$	$0.872 \pm 0.034$	$0.874 \pm 0.034$	$-0.692 \pm 0.075$
18.00- 6.00	—	$0.576 \pm 0.096$	$0.517 \pm 0.105$	$0.801 \pm 0.052$	$-0.384 \pm 0.123$

surface temperature in the daytime during the period extending from the 6th hour to the 18th hour are larger than 0.9, and therefore we may recognize these two factors as being very effective upon the activity of the egg-laying. The evaporation and the air temperature are noted as being the next factors showing a similar degree such as 0.874 and 0.872 of coefficients.

The humidity may not be recognized as an effective factor though there can be seen a negative correlation.

ii) During the period extending from the 6th hour to the 10th hour, the evaporation and the total radiant energy are most remarkable as having a coefficient of correlation respectively 0.928 and 0.884. The coefficient of correlation is 0.822 in the case of the air temperature. But the soil surface temperature and the humidity have no close relation with this activity.

From the above facts it is clear that in the part of the daytime above mentioned the evaporation and the total radiant energy are the most effective factors strongly controlling the activity of the egg-laying.

At about noon, during the period from the 10th to the 14th hour, the soil surface temperature which was not very noticeable in the morning becomes a remarkable factor, the coefficient of correlation being indeed 0.932. At the same time great values of coefficients of correlation were obtained also in the cases of the evaporation, the air temperature and the total radiant energy. But the coefficients of these four factors have such small differences among them that the correlations are recognized in a similar degree. As mentioned in the following, though the value of the coefficient of the evaporation may be great, its biological meaning is not



noticeably great, since this value was obtained only by a parallel phenomenon caused by other meteorological factors such as the total radiant energy, the soil surface temperature and the air temperature.

In the period extending from the 14th hour to the 18th hour, the soil surface temperature and the air temperature have each a close correlation showing respectively 0.839 and 0.825 in the coefficients of correlation. The total radiant energy is next in importance, but the humidity is not again effective in this case. In conclusion in this period the soil surface temperature and the air temperature measured at the height of 30 cm. above the surface of the ground form the main controlling factors.

iii) During the night there are no remarkable climatic factors controlling the activity of the egg-laying, except the evaporation whose coefficient of correlation is 0.801,

In conclusion, we may say judging from the investigation of the coefficients of correlation that the total radiant energy and the soil surface temperature are the most important factors controlling the egg-laying activity of this weevil in the daytime, while in the night the evaporation becomes important. In the morning, in the period from the 6th hour to the 10th, the evaporation and the radiant energy are the most important factors controlling the said activity, but in the course of time the degree of correlation of the evaporation decreases gradually, while that of the soil surface temperature and of the air temperature increases. Then in the evening from the 14th hour to the 18th, the soil surface temperature and the air temperature become most noticeable as the controlling factors.

Now from Figures 5 and 6 it is evident that rainfall inhibits almost all of the activity of egg-laying. We find only a small number of eggs laid on a rainy day. This may be clearly seen in the cases of such days as the 17th, 19th, 23rd, 24th and 31st days of May.

#### DISCUSSION

There are a fairly large number of investigations which have been made concerning the correlation between the climate and the activities of insects.

Recently NASH (1933) has recognized an interesting relation existing between the climate and the density of the Tsetse Fly. According to NASH, the air temperature itself does not affect the density of the fly, but the evaporation and the saturation deficiency are closely related to its density showing  $-0.729$  and  $-0.794$  respectively in the value of coefficients of correlation. This is the reason why the flies occur abundantly during

the wet season and are rare during the dry season. It may be recognized from the above investigation that the dry or wet condition of the climate has a remarkable influence upon the density of the occurrence of this insect.

In other cases, YOTHERS (1927) discussed the existence of a close parallel correlation between the daily mean air temperature and the number of the Codling Moth captured by traps. According to his opinion, the Codling Moth is not attracted to bait when it is cool, but when the daily mean temperature goes up to 70°F. or above, they are strongly attracted to the bait and are abundantly captured by traps. Also in the case of the Alfalfa Weevil, it is shown by PARKS (1914) that the progression of the activity in the oviposition is strongly affected by the mean daily temperature, while in the case of the Codling Moth GARRET (1923) shows that the same activity is influenced by the mean evening temperature. In the cases of Grasshoppers, *Melanoplus mexicanus mexicanus* SAUSSURE and *Camnula pellucida* SCUDDER, it was observed by PARKER (1930) that their activities in general and the power of egg-production in particular are controlled to a large extent by the air temperature and the soil surface temperature. The favorable temperature for egg-production in the case of these grasshoppers is similar to that for general activities. Thus in *Melanoplus mexicanus mexicanus* SAUSSURE the egg-laying is never observed when the air temperature is below 69°F. and the soil surface temperature is below 98°F. But this insect begins to lay eggs when the air temperature and the soil surface temperature become 71.1°F and 99.8°F. respectively and it continues till the former goes up to 80°F. and the latter up to 107°F. According to BODENHEIMER and KLEIN (1930) the activity of the "Ernte Ameise" (*Messor senirufus* E. ANDRE) is greatly affected by the soil surface temperature, so it is most active during the period extending from April to June, but it becomes inactive in summer and then is again active in autumn and inactive in winter. As the favorable temperature ranges from 17°C. to 19°C., when we observe its diurnal activity it is active respectively at "Mittagsstunden" in winter, "Abendstunden" in spring, "ganzen Nacht" in summer and "erste Nachthälfte" in autumn.

From the above mentioned facts, it is evident that in the case of an insect which lives on surface of the soil or close to the same its activity is strongly affected in general by the soil surface temperature, though the main climatic factor which controls the activity of the insect may vary according to the kinds of insects and their environmental conditions.

In spite of this evidence that solar radiation may directly controll the

activity of various kinds of insects, it seems to have been neglected in the case of ecological investigations. It is generally well known that most insects are very active in sunshine, and also that this activity depends mainly upon direct solar radiant energy. The fact, that the activity of oviposition of the Strawberry Weevil keeps a close correlation with the total radiant energy as well as with the soil surface temperature in nearly perfect correlation showing a value of above 0.9 in the coefficient of correlation, may be enough to prove the great influence of the solar radiant energy upon this kind of activity of the Strawberry Weevil.

Now from the point of view of the correlation between the total radiant energy and the activity of the weevil and of the correlation between the soil surface temperature and the same, the ecological investigation on the body temperature of this weevil which I am going to try in the near future is naturally expected.

All the weevils observed in the present experiments were those living among the low strawberry plants grown close to the soil surface, but in the case of those living among the wild shrubberies such as raspberry plants, their environment may be almost outside of the influence of radiation from the soil surface, and thus the activity of egg-laying will be controlled mainly by the total radiant energy.

During the period extending from the 10th hour to the 14th hour, the soil surface temperature and the air temperature which have not been noticed before that period take precedence over other factors controlling effectively the egg-laying activity of this insect. This fact depends mainly upon the following phenomenon that the soil surface temperature acts in company with the air temperature upon the activity, both being raised by solar radiation and by convection. This tendency comes to a climax in the afternoon, and the coefficient of correlation of the soil surface temperature is greatest at this time when it is compared with those of the others.

The climatic condition of the night is quite different from that of the daytime and the most remarkable correlation is shown by the evaporation. In this case it may be considered that the evaporation controls the activity of this insect only mechanically; namely the relative water content of the air increases in the night in company with the fall of the air temperature, and accordingly the weevils influenced by the wet condition of the air become extremely inactive and are often even embedded in some water drops attached to the strawberry plants. Therefore the weevil is active only while it is keeping away from the dewpoint. It may also be seen that

a similar climatic condition which lasts until morning extending from the 6th hour to the 10th will control the activity of the egg-laying. Therefore the degree of the correlation of the evaporation is great in the morning. Since we have learned the influence of the evaporation upon the activity of the weevil, it seems to be rather reasonable to assume that the great value of the coefficient of correlation of the evaporation in the daytime is mainly due to the parallel phenomenon accompanied by other climatic factors, and that the evaporation on this occasion is accordingly not a factor to be considered.

Briefly the diurnal activity of the egg-laying of the Strawberry Weevil has a close correlation to climatic change. In the daytime the total radiant energy and the soil surface temperature are the main factors effective in controlling the said activity of this weevil. Hence we can not fail to recognize the importance of the solar radiation together with the soil surface temperature. Now in this case it must be noticed that the solar radiation is strongest at noon, but the soil surface temperature attains its climax at about the 14th hour. From these facts it must be clear that the oviposition is most actively executed at about noon and is more active in the afternoon than in the morning.

The solar radiation controls the said activity strongly in the morning and at about noon, and the soil surface temperature and the air temperature become noticeable at about noon and in the evening.

During the night the evaporation is most remarkable, and even in the morning is still effective.

Rainfall may be considered as a limiting factor for the egg-laying activity, as we do not find the flower-buds injured on rainy days; and often we have observed weevils embedded in rain drops attached to the strawberry plants. In such cases the solar radiant energy after a rainfall does not affect the oviposition in the usual way, but rather is used up in the evaporation of water. Accordingly it is conceivable that rainfall has the effect of lowering the degree of the correlation of the radiant energy.

#### SUMMARY

1) The present paper deals with results obtained by the statistical investigation which was made on the correlation between the climate and the diurnal activity of oviposition in the case of the Strawberry Weevil.

2) As climatic factors controlling the activity of the egg-laying of the said weevil we have chosen the following six, viz., i) the total radiant energy, ii) the soil surface temperature, iii) the air temperature measured

at the height of 30 cm. above the soil surface, iv) the evaporation, v) the humidity, and vi) the rainfall.

3) Referring to the diurnal activity of egg-laying of the Strawberry Weevil we know that 88.4 percent of the total number of eggs laid daily are deposited in the daytime and only 11.6 percent of the same are laid in the night. It is known again that the activity is most active at about noon and is more active in the afternoon than in the morning.

4) The activity of oviposition of this weevil is controlled mainly by the total radiant energy and by the soil surface temperature during the daytime, while a distinct correlation between the said activity and the evaporation is noticeable during the night.

5) When we investigate the progression of the correlation in the course of one day, we know that the evaporation and the total radiant energy are most effective in the morning during the period from the 6th hour to the 10th, also that the total radiant energy, the soil surface temperature and the air temperature all act strongly at about noon during the time from the 10th hour to the 14th hour and lastly that the soil surface temperature and the air temperature are most effective in the afternoon during the period extending from the 14th hour to the 18th.

Of the six factors above alluded to it may be conclusively recognized that the total radiant energy and the soil surface temperature are most noticeable, the former being most effective in the period extending from the 6th hour to the 14th and the latter being most prominent in the period extending from the 10th hour to 18th.

6) The rainfall inhibits almost all of the egg-laying activity of this weevil.

#### ACKNOWLEDGMENT

I wish here to express my great thanks to Professor Dr. SANJI HÔZAWA who gave me kind instruction and encouragement in the course of my investigation. I am also greatly indebted to Assist. Professor Dr. ISAO MOTOMURA for his kind and valuable suggestions given me in the progression of the present investigation. I wish here to take this opportunity of thanking Dr. YOSHIJI YOSHII, Professor of Plant Ecology of the Botanical Institute of the Tôhoku Imperial University, Dr. SHOJI KONDO, Professor of the Medical Department of the Tôhoku Imperial University, and Dr. KOKI ABE, Assist. Professor of the same, all of whom have given me much assistance in using the meteorological instruments.

I am also grateful to Mr. TADASHI MIYAZAYA, the owner of the Straw-

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# ON THE BARBELS OF A JAPANESE SEA CATFISH, *PLOTOSUS ANGUILLARIS* (LACÉPÈDE)

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(With three figures)

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## INTRODUCTION

This catfish is commonly found in the shallow water along the coast of the southern Japan. It is a sleek fish covered with smooth skin, striped with yellow, and armed with sharp pectoral and dorsal spines which render it a very disagreeable object to fishermen. It is frequently observed during the summer time that the fishes group together into a more or less spherical mass-formation and swim slowly near the surface of the bottom of a sandy bay. This fish has four pairs of barbels about the mouth: the nasal and maxillary barbels are each one pair and the mental ones are two pairs, and all of them are almost equal in length, 13-25 mm. in the adult (Fig. 3).

The barbels of American catfishes have been studied by such authors as HERRICK (1901, 1903), MAY (1925), OLMSTED (1920) and WRIGHT (1884). A Japanese catfish, *Parasilurus asotus* L. has been observed by ATODA (1935), but I am aware of no reports previously been published regarding the barbels of this sea catfish. The present paper has been done in an attempt to observe the barbels of this fish histologically and experimentally.

Here I desire to acknowledge my sincere thanks to Prof. S. HATAI for his valuable suggestion and kind guidance throughout the course of this work, and my thanks also due to Mr. TAKANAGA MITSUI for affording me all possible facilities in pursuing the present investigation.

## MATERIAL AND METHOD

The material employed in the present observations was collected from the bay in front of the Institute. For fixing of the barbels for histological purpose, Bouin's fluid, Zenker's fluid and formol-Zenker were tried. For staining, Delafield's haematoxylin with eosin, Heidenhain's iron-haematoxylin



with orange G. and Mallory's triple connective tissue staining mixture were used. Golgi's method was used also and for nerves alone osmic acid used.

The operations on the olfactory organs and eyes were performed: the olfactory nerve was cut by incision posterior to the nasal barbel and the eyes were removed completely. For operation the fishes were anaesthetized in 10 per cent solution of ether in sea-water.

#### HISTOLOGICAL OBSERVATIONS

The eight barbels of this fish are similar in form and structure, somewhat depressed laterally, and the basal portion is much broader than the distal one. The majority of the epidermis, the outer layer, of the barbel is composed of stratified epithelium among which the cutaneous taste buds are found (Fig. 1). These buds lie closer in the epidermis of the distal edge than in that of proximal one. This concentration of the taste buds on the distal edge is expected, since it is this portion of the barbel which touches objects in the search of food. The taste buds flush with the surface or even project beyond the level of the outer epidermal cells, and the apices of these are not covered with cuticle or hair. They are not depressed, nor do they lie at the bottom of a pit as do the three types of sense organs belonging to acustico-lateral system. The buds are pear-shaped (Fig. 2) and less spherical than those found in the goat-fish, *Upeneoides bensasi* (T. & S.). The sense cells composing the taste bud are arranged regularly side by side, and are situated upon the papillary eminence of the dermis which is raised under the organ. This intervening space is filled with nerve fibres which lose their myelin sheath. The individual sense cell is very long and possesses a distinct bulge, about one-fourth of the length of the cell from the proximal end, to accommodate the heavily staining oval nucleus. The distal end of the sense cell terminates in a single short process, and the proximal end of the cell may terminate either in a single nerve or it may be branched.

Besides the taste buds, there are found also the pigment cells and club-cells among the epidermal cells.

The inner layer of the barbel, the dermis, consists of layers of fibrous connective tissue among which the blood vessels and melanophores are found, but the larger part of it is occupied by a large nerve trunk. This trunk is a branch of the V+VII cranial nerve and divides in two in a barbel. In its course from the base of the barbel to its tip, numerous

side branches are given off for some distance through the connective tissue, reaching to the base of the dermal papillae, where the taste buds

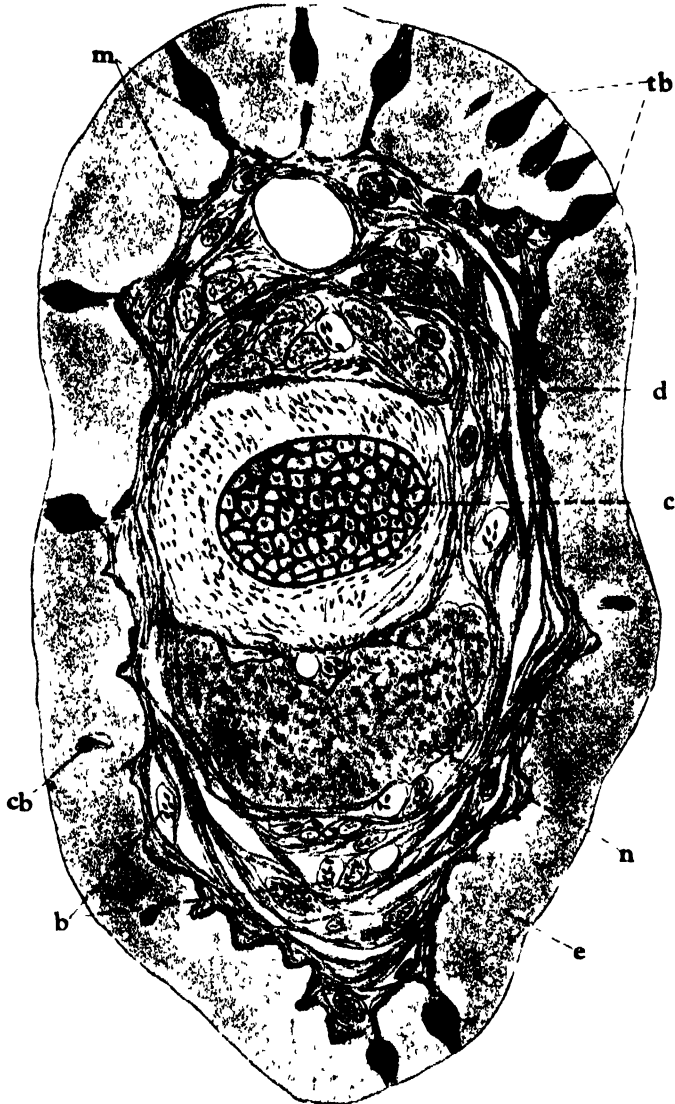


Fig. 1. Cross section of the barbel, showing diagrammatically its structure.  $\times 70$ . b, blood vessels; c, rod of cartilage; cb, club-cell; d, dermis; e, epidermis; m, melanophores; n, bundles of nerve fibres; tb, cutaneous taste buds.

are found. At this point, however, the nerves lose their sheath and continue as naked fibres. In the central core of the dermis, a rod of



Fig 2. Transverse section through the cutaneous taste buds which are unbedded among the epidermal cells  $\times 100$ .

cartilage is situated and it stretches from the base of the barbel to its distal end, in parallel with the nerve trunk.

#### EXPERIMENTAL OBSERVATIONS

From the histological observations, it was suspected that the barbel of this fish may function similarly to that of the Japanese goatfish, *Upeneoides benasasi* (SATÔ, 1936 a, b). In order to ascertain my suspicion, the reaction of this fish to its bait was observed.

The experimental observations were made upon twenty fishes which were kept in the aquarium of normal sea-water ( $1 \times 1 \times 1.5 \text{ m}^3$ ). This fish is a bottom and nocturnal feeder; strongly thigmotactic, always resting with as many points of the body as possible on the substratum and swims slowly in a spherical mass-formation in the aquarium. When lugworms were carefully dropped into the aquarium, the fish was unable to find its meat so quickly as other fishes do; in other words, no attempt was made

to use the sense of sight in feeding. And moreover, this fish does not trail food substance with his barbels so actively as the goatfish does, but he only touch it with his barbels or lips passively. He generally seized the worm after his barbels or lips came into contact with it. This observation suggests that the eye is probably of little importance in feeding in this fish, as in American catfishes (HERRICK, 1902; OLMSTED, 1918, PARKER, 1910). So, at first, I have ascertained the part played by the eye in cognition of food. Five fishes were eliminated of their power of vision and then observed. These fishes swam normally excepting that they occasionally collided with the walls of the aquarium and did not aggregate, though they would sometimes turn and follow another fish when closely passing by the other. If a fresh lugworm was dropped, these operated fishes detected it by touching it with their barbels or lips in an essentially normal way. From this test, it is clear that the ability to recognize food is not affected by the elimination of the vision.

Next, I observed the reactions of the normal fishes to two wads of white cheese cloth, one of these containing lugworms and the other with stone. They were observed ten minutes, and a record was taken of the number of times each wad was bitten by the fish. This test was performed on the same fishes for the succeeding two days and with similar results, as are shown in the following table.

TABLE 1.  
*Number of times the two wads were bitten by fifteen  
normal fishes during each ten minutes.*

	Wad with lugworms	Wad with stone
1	9	0
2	6	1
3	5	0
4	5	0
5	6	0
6	5	0

It is perfectly clear from the above table that the fish is able to sense the difference between the wad of cloth with worms and that with stone. Next I repeated this test on five fishes whose barbels were cut at the base with a sharp scissors. The operated fish soon swam normally and still aggregated. When lugworms were dropped into the aquarium, these fishes were able to detect the bait by the aid of their lips in contact with them. One day after operation, the reactions of the fishes to two wads

as mentioned above were observed. The result was that the loss of ability to distinguish two wads was not caused by removing the barbels, in strong contrast with the case of the goatfish.

If so, what sense organs are most concerned with the reaction just described above? PARKER (1910) who studied the olfactory reaction of an American catfish, *Amiurus nebulosus*, concluded that the olfactory apparatus of the catfish serves in sensing food at a distance beyond that at which the taste organs are capable of acting. In this sea catfish, too, is the olfactory organ most valuable in cognition of food? To ascertain this question, the reactions of five fishes whose olfactory tracts were cut to their food were observed. The aperture of the nasal chamber of this fish is located immediately posterior to the root of the nasal barbel and is slit-like in form. The operated fishes swam as usual and the aggregating habit was not at all affected by this operation. These fishes detected their food, although they took more time than the normal ones which sense their food. Moreover, they were able to distinguish one wad with meat from the other with nothing. From this observation, it may be believed that this fish is not only able to recognize its food through the olfactory apparatus, but also by the use of other sense organs. The olfactory sense, however, seems to be useful to this fish in procuring food, since the operated fish took much more time than the normal one in finding its food on the one hand, and that the normal fish seldom shows excitement before touching food with his barbels or lips on the other. About one hour after the completion of the test just described, their eyes and barbels were removed in an attempt to ascertain whether or not these five fishes are able to recognize their food in the absence of those organs. These operated fishes swam normally, excepting that they occasionally collided with the wall and failed to swim in formation of aggregation as usual. The following tests were performed at the end of three days after this operation. As the lugworms were given, these operated fishes never failed to recognize their food which fell to the bottom, although they spent more time compared than the normal ones in sensing their food. In addition, this experiment showed that they could also distinguish one wad with meat from the other without any.

As for the last test, an experiment was planned to ascertain whether or not this fish is able to recognize the location of food through a chemical sense, judging from the reaction of the fish to two wads. A wisp of cotton attached to a hooked end of a wire was carefully lowered into the water, and brought near a fish. At no time did this fish pay

any attention nor show any excitation, but avoided and turned from it. If, however, the cotton saturated in the juice obtained from the lugworms was used in place of the cotton without any such a treatment, it was pursued and taken into the mouth by fish as if it was meat, though after about ten minutes the fish took no further notice of it, probably due to a complete diffusion of the juice into the water. This experiment was repeated with similar results. Moreover, this fish showed the normal reaction to this test even when its eyes and barbels were removed and olfactory tracts cut.

These observations show, beyond doubt, that this catfish obtains its food through the use of a chemical sense; in other words, this fish responds to a stimulation on its olfactory apparatus and external gustatory organs, barbels and lips, by substances in dilute solution emanating from its meat. This fish, therefore, seems to discover the hidden food by the sense of smell and taste, though this point needs further investigation.

#### CONSIDERATION AND CONCLUSION

This fish is a bottom feeder possessing fair power of sight and has eight barbels about the mouth. This fish, however, does not move its barbels so actively in trailing of food as the goatfish does, but generally detects the meat after its barbels or lips have come into contact with the meat. From Table 1, it is clear that the normal fish is able to sense the wad with meat from the other without any. This ability is not at all influenced by the removing its barbels, in strong contrast with the case of the goatfish in which such an operated fish is unable to do so. Further, this is not disturbed by the elimination of the olfactory sense, differing from *Amiurus nebulosus*, in which such an operated fish made no attempt to distinguish these two wads (PARKER, 1910). Additionally to this, the elimination of the vision leads the destruction of the aggregation as shown by BOWEN (1931) in *Ameiurus melas*, but the ability to sense its food is not hindered by this. This difference in characteristic reaction of the goatfish and sea catfish is in my opinion cleared fully from the following facts. In the goatfish, the cutaneous taste buds is found only on the outer surface of the barbel, and so the removing of the barbels leads to the loss of the external gustatory organs wholly. In the catfish, however, this buds are located not only on the barbels, but also on the general surface of the body, especially on the lips. The lips of this fish are provided with the epidermal foldings (Fig. 3), on

which the majority of the taste buds are located. In this catfish, therefore, the elimination of all barbels causes a part of the external gustatory organs to become functionless.

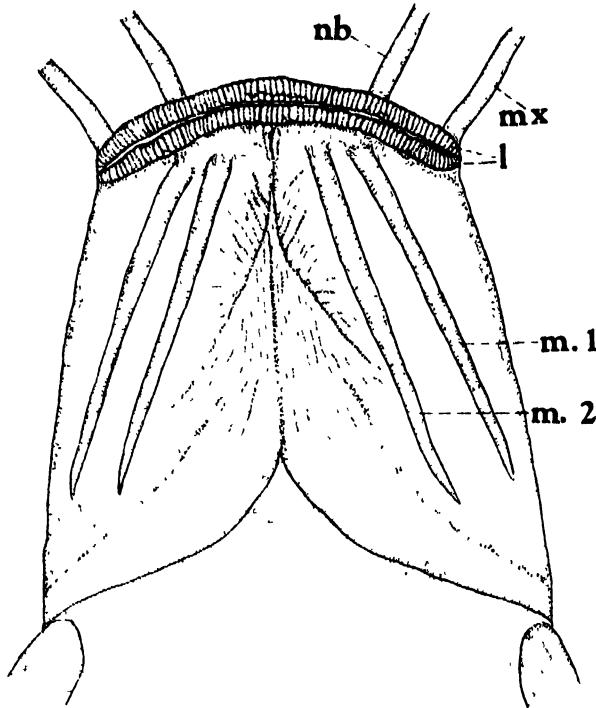


Fig. 3. Ventral view of the head, showing the barbels and lips.  
l. upper and lower lips; m. 1 and m. 2. 1st and 2nd mental barbel;  
mx. maxillary barbel; nb. nasal barbel.

That the fish recognize its food through the chemical sense is made clear from the results of my test. And, the olfactory organs and barbels seem to be most important for this reaction. Because the fish whose barbels or olfactory sense are eliminated takes much time compared with the normal one in sensing its food. The lips of this fish are also valuable in detecting of food, since this fish never fails to detect its food even when the vision, olfactory sense and barbels are all eliminated. The barbels, in this case too, seem to be useful to this fish in recognition of food as like as that of the goatfish, but they are probably not so important in its daily activity as in the case of the goatfish.

From these results, just summarized above, I am inclined to think that this fish detects its food through both the sense of smell and taste.

## SUMMARY

1. This fish possesses eight barbels about the mouth with the same form and structure. Among the epidermal cells of the barbels, the cutaneous taste buds are imbedded. The taste bud consists of the sensory cells which are very long cells arranged regularly side by side, and are situated upon the papillar eminence of the dermis.

2. A branch of the V + VII cranial nerve enters each of barbels and divides in two in a barbel, and in its courses from the base of the barbel to the tip, the side branches are given off numerously and their ends terminate to the dermal papillae at whose apices the taste buds are found. In the central core of the dermis, a rod of cartilage is found. This rod stretches from the base of the barbel to its distal end, in parallel with the nerve trunk.

3. This fish recognizes and determines the location of food substances through a chemical sense. This power is not lost even when the olfactory sense and vision are eliminated and barbels are all removed.

4. In this catfish, its olfactory apparatus and external gustatory organs, especially barbels and lips, seem to serve as receptor for the chemical sense.

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# PRELIMINARY SURVEY OF THE EARTHWORMS OF QUELPART ISLAND

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(With five figures)

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From Quelpart Island no earthworms have hitherto been known, thus in order to find what kind are represented, I made a trip for collection of earthworms to this island, in August of 1934. The materials obtained, comprise only sixty-six specimens belonging to two genera and five species, namely; *Drawida anchingiana* CHEN (*Dr. gisti* var. *anchingiana* CHEN), *Pheretima masatacae* (BEDDARD), *Ph. quelparta*, n. sp., *Ph. kanrazana* var. *typica*, n. sp., n. var., and *Ph. kanrazana* var. *increta*, n. var. This island may be unfavourable to the distribution of the earthworms since it consists largely of the Tertiary volcanic rocks, its surface is mostly very rough and with little humous and with black volcanic pebbles and masses of decomposing lava. But, more species will be probably found by further researches.

It was much interesting to find *Ph. masatacae*, a species which has not been re-collected nor re-examined by any one since BEDDARD's original report in 1892, and *Dr. anchingiana*, an interesting worm first recorded by CHEN in 1933 from Anhwei and Kiangsu, both were found to be rather widely distributed on this island but small in number.

It is a pleasure to record here a debt of gratitude to Dr. SHINKISHI HATAI, professor of the Tôhoku Imperial University, for his serial guidance to my study; and to Dr. HARUJIRÔ KOBAYASHI and Dr. TAMEZÔ MORI, professors of the Keijo Imperial University, for their kind direction and encouragement given me throughout this work.

## *Drawida anchingiana* CHEN

1933 *Drawida gisti* var. *anchingiana*, CHEN in: Contr. Biol. Lab. Sci. Soc. China, Vol. IX, Zool. Ser., No. 6, pp. 202-203, fig. 6.

Saishû, Japanese garden where grass-plots are roughly planted, one matured, two semi-matured, and one juvenile specimens.

Seikiho, gutter side, one semi-matured and one juvenile specimens.

Mt. Kanra, mountain path side, near Saishû at about 1,000 M in altitude, one semi-matured and one juvenile specimens.

*Description :*

*External characteristics :* Length 56–68 mm, diameter 4–4.5 mm, number of segments 125–132. Prostomium prolobous. Middorsal line thinner, appearing to be dark blue behind clitellar region, along its non-functional dorsal pore-like depressions found behind 2/3 caudalwards. Colour, dark greenish blue dorsally, light greenish ventrally, clitellum fleshy.

Clitellum distinct in matured specimen, in X–XIII, not swollen, but clearly differentiated dorsally from the neighbouring segments in glandulation and in colour.

Setae may be stouter than *Dr. gisti*, but their arrangement is similar to the latter. Those on II not weakly built but slightly smaller than the rest; no marked difference in size between anterior and posterior segments to the clitellum distinguishable;  $ab=cd$ , in general  $aa$  narrower than  $bc$ , but greater on II–IV or V,  $dd$ =about 4/7 of the circumference.

Nephridiopores in line with  $c$ .

In all of the specimens at hand, penes are wholly everted out. Penis in intersegmental furrow of 10/11, between  $b$  and  $c$ , much nearer to  $c$ , or nearly closing to  $c$  line with its basal lateral margin, surrounded by swollen skin, conical in shape, not sharply pointed distally, rounded at base, about 0.6 mm in total length and 0.4 mm in basal width, with a minute primary pore situated at its distal end (Fig. 1. A).

Female pores, one pair, in line with  $b$ , rather distinct as transverse slits at the anterior border of XII.

Spermathecal pores, one pair, in intersegmental furrow of 7/8, each represented as a large transverse slit, just medial to  $c$ . In one case, a small papilla was found posteriorly to the pore being slightly sunken into the spermathecal aperture; but such papillae are usually invisible externally even if accessory glands corresponding to those are placed internally.

Genital papillae circular, with a pigmented glandulated center, distinctly elevated, usually surround by swollen skin. In five specimens they are located as follows:

Locality	No.	Right side	Left side
Saishû	1	X, medial to $a$ , postsetal	0
		XI, medial to $a$ , presetal	0
	2	X, medial to $c$ , postsetal	X, medial to $c$ , postsetal
	3	VIII, medial to $c$ , setal line	VIII, medial to $c$ , setal line
		X, medial to $c$ , postsetal	X, medial to $c$ , presetal

Seikiho	4	VIII, medial to c, postsetal X, medial to c, presetal	VIII, medial to c, postsetal X, medial to c, presetal
Mt. Kanra	5	VIII, medial to c, presetal X, medial to c, postsetal	VIII, medial to c, presetal X, medial to c, postsetal

### *Internal anatomy:*

Septa 5/6 thick, 6/7-8/9 very much thickened, the rest thin and membranous; 9/10 shoved to middle part of X, 10/11 to anterior part of XII to meet 11/12 forming ovarian chamber, 12/13 to anterior part of XIII, the posterior ones resuming nearly normal attachment.

Gizzards, three in all specimens, in XII-XVI or XI-XVI; in one specimen a thin walled rudimentary one found in XI which may develop to make them four in number.

Hearts, four pairs in VI-IX, relatively stout.

Testis sacs, one pair, moderate in size, ovoidal, about  $2.2 \times 1.6$  mm, filled with yellowish sexual products, suspended on 9/10 placing nearly equal parts in IX and X without constrictions there. Sperm-ducts rather fine, moderately long, formed of two small masses of coils on both sides of 9/10, finally entering into ental end of prostate.

Prostates small, cylindrical, smooth on surface, usually slightly curved in its middle part, longest about 2.2 mm, ental end usually larger and roundish, about 1 mm in width, irregularly placed under intestine or ovarian chamber, ectal end rather slender, passing through body wall to penis which is wholly everted.

Ovarian chamber formed by septa 10/11 and 11/12 which meet merely on dorsal side and are rather widely separated on ventral side from each

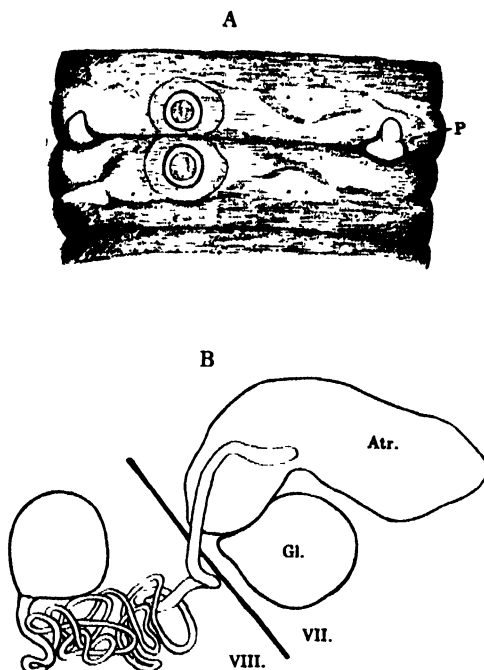


Fig. 1. *Drawida anchingiana*. A. Ventral view of X-XII, ca.  $\times 12$ , P. penis. B. Spermatheca with duct and atrium, ca.  $\times 25$ , Atr. atrium; Gl. accessory gland which is often associated with spermatheca.

other. In matured specimen, egg sacs are fully filled with yellowish mass of ova, considerably long extending posteriorly to XVI or XVII and reaching to about XX if stretched, anterior half blade-shaped, situated on both sides of gizzards, posterior half slender and rod-like but not moniliformed as in *Dr. japonica*, simply twisted and situated under gizzards or intestine. Total length of sac about 7 mm. Ova small, 3–6  $\mu$  in diameter.

Spermathecal atrium cylindrical, usually slightly curved at ectal third, closely resembling prostate in shape, largest about 1.6 mm in length and 0.6 mm in width, always situated in VII; ampulla small, thin-walled and spherical, about 0.7 mm in diameter, with its long duct always situated on posterior face of 7/8; duct arising from the lower side of the ampulla, not sharply marked off from the latter, entally making a number of great coils and ectally passing through septum 7/8 into VII with gradual increase in thickness and finally entering into posterior ectal third of the atrium. A large spherical gland which is nearly equal to, or rather larger than, the spermathecal ampulla, often associated with ectal end of antrium (Fig. 1. B.).

Large urn-shaped accessory glands found inside corresponding to the external genital papillae.

*Remarks :*

The present species was first recorded by CHEN ('33) as a variety of *Dr. gisti* (CHEN, '33) on the specimens collected from Anhwei and Kiangsu. But, I think it is more advisable to classify it as a species distinct from *Dr. gisti*. First reason for such treatment is that CHEN's *Dr. gisti f. typica* differs from MICHAELSEN's *Dr. gisti* in some important characters, as already pointed out by GATES ('35). Classifying the present species as a variety of CHEN's species would cause some systematic confusions, until both MICHAELSEN's and CHEN's species are re-examined whether they are specifically identical or not. Secondly, the present species differs from both MICHAELSEN's and CHEN's *Dr. gisti* in several characters which seem to be worthy of specific separation from them.

The differences from MICHAELSEN's *Dr. gisti* are as follows: the number of segments, position of apertures of penial chambers, limitation of penial chamber to body wall, texture and size of prostate, position of spermathecal atrium, and connecting point of the spermathecal duct with its atrium. Number of segments 134–145 (Chinese specimens) and 125–132 (Korean) in the present species, 180–190 in the latter. In all of the present specimens at hand, as the penes are wholly everted, the limitation

of penial chamber to the body wall was not satisfactorily examinable. But, from the descriptions and illustrations given by MICHAELSEN for *Dr. gisti* and by CHEN for the present species clearly show the difference of this character, i.e. deep in the former and shallow in the latter. Apertures of penial chambers or penes are situated "nearer to b" in *Dr. gisti*, but "much nearer to c" in the present species. CHEN's Anhwei and Kiangsu specimens may, as GATES remarked on them, not be ~~sexually~~ matured. Except for one specimen, ~~all of mine~~ are also not sexually matured. In these ~~specimens~~, as the main differences from the matured one, the following were observed, the emptiness and absence of posterior ~~slender~~ appendix of ovisac, and indistinctness of clitellum. Small size of the spermathecal ampullae, and small size and absence of granulation of or smooth surface of the prostates may not be due to their immaturity, but rather I think, may be specific characters for the present species; or, at least, the surface of the prostates may not be as warty as that of *Dr. gisti*. In the latter species the spermathecal duct "opens into the broad distal end of a large long sac-like spermathecal atrium", but in the present species "enters into posterior ectal third of the atrium". Spermathecal atrium is situated in VII rather than VIII; that is an unique character for the present species.

The present species may be a widely distributed form on this small volcanic island, though not so abundant as *Ph. quelparta*.

### *Pheretima masatakae* (BEDDARD)

1892, *Perichaeta masatakae*, BEDDARD in: Zool. Jahrb. Syst. Geogr. Biol., Bd. 6, p. 761. Seikiho, hill side, two clitellate specimens (one of these broken at the clitellar region).

#### *Description:*

*External characteristics:* Length 114 mm, greatest diameter 6.5 mm, number of segments 117. Colour, dorsally dark brown, ventrally yellowish brown, clitellum light russet. Prostomium, epilobous ca. 2/3. First dorsal pore in 11/12, distinct and functional.

Setae beginning on II and rather large; both mid-dorsal and -ventral breaks slight, aa=1.2-1.9 ab, zz=1.4 2.2 zy; setae on II-IX and on several segments of the posterior end enlarged and vaguely irregularly interrupted, the rest of the body nearly equal in size; ventral ones slightly larger and more widely spaced than the dorsal. Setal number as follows: 23/III, 31/IV, 42/VII, 43/VIII, 49/IX, 50/XII, 64/XVII, 62/XX, 59/XXX, 12 (VII), 13 (VIII), 14 (IX) between spermathecal pores, 14 between male pores.

Clitellum entire, in XIV–XVI, without setae.

Male pores ventrolaterally on the setal line of XVIII, about  $\frac{1}{3}$  of the circumference apart; each on a small oval papilla placed on lateral part of circular, apparently glandulated and slightly elevated area with a few incomplete circumferential furrows. On each area, medially to the male porophore two circular genital papillae with central depression present, one presetal and the other postsetal; on right side only similar one found presetally on XIX in line with the former (Fig. 2. A.).

Female pore single, on XIV midventrally.

Spermathecal pores, moderately sized, two pairs in intersegmental furrows of  $\frac{7}{8}$  and  $\frac{8}{9}$ , about  $\frac{2}{5}$  of the circumference ventrally apart. Just medially to each pore two small genital papillae found on both borders of the intersegmental furrow as facing each other. Each papilla much smaller than those of the male segment, conical in shape, with a minute opening on its tip; similar ones were also found on  $\frac{9}{10}$  in line with the former (right side only) (Fig. 2. A and B.).

*Internal anatomy:*

Septum generally much thickened,  $\frac{8}{9}$  ventrally traceable,  $\frac{9}{10}$  absent,  $\frac{5}{6}$ – $\frac{7}{8}$  and  $\frac{10}{11}$ – $\frac{12}{13}$  much or very much thickened and muscled,  $\frac{13}{14}$  slightly thickened.

Nephridial tufts, moderately thick, in V and VI.

Gizzard, large relatively to the body size, globular in shape. Intestine beginning to swell in XV. Intestinal caeca, simple, originating in XXVI, extending as far anteriorly as XXII, each with several serriformed outgrowths on ventral margin only.

Hearts, four pairs in X–XIII, moderate in calibre; dorsal vessel rather large. Paired lymph glands found along dorsal vessel behind  $\frac{15}{16}$  caudalwards.

Testis sacs, two pairs in X and XI, rather small; anterior pair oval in shape, connected ventromedially with each other forming a low V-shape, posterior pair larger than the anterior, fused with its fellow forming a transverse sac. Both sacs are separated from each other by thick septum  $\frac{10}{11}$ . Seminal vesicles, small, two pairs in XI and XII, somewhat darkened and weakly vesicular on surface; anterior pair smaller than the posterior; each with a distinct dorsal lobe, whitish and almost smooth on surface. Testes and funnels usual in position and structure. Spermathecae on each side meeting in XIII. Pseudovesicles, one pair, rather large, attached to the posterior face of  $\frac{12}{13}$ .

Ovaries moderate in size, situated in usual position.

Prostatic glandular portion absent; duct very short, thin but muscled, shining on surface, nearly straight or simply twisted, with nearly equal thickness throughout. Close and medially to the ectal end of the duct two large accessory glands with long stalk found corresponding to the external papillae (Fig. 2. C.).

Spermathecae very small, in VIII and IX; ampulla round or ovoid, about 1 mm in diameter; duct short but thick, distinctly marked off from the former; diverticulum slightly longer than the main portion, its ental half distended as a cylindrical seminal chamber, the ectal half slender. Close to the ectal end of each spermathecal duct present two relatively large accessory glands with moderately long stalk; saccular portions of these glands on each side appear to be arranged with nearly equal distance from one another (Fig. 2. D.).

#### Remarks:

*Perichaeta rokugo*, *P. nipponica*, *P. masatacae*,

and *P. tokioensis* were erected as new species by BEDDARD for specimens collected by MASATAKA ROKUGO from unknown places in Japan. Of these, *P. rokugo* is synonymous with *Ph. hilgendorfi*, and *P. nipponica* with *Ph. heterochaeta*. *P. masatacae* like *P. tokioensis* have not been re-examined or re-collected since the publication of BEDDARD's paper. His original

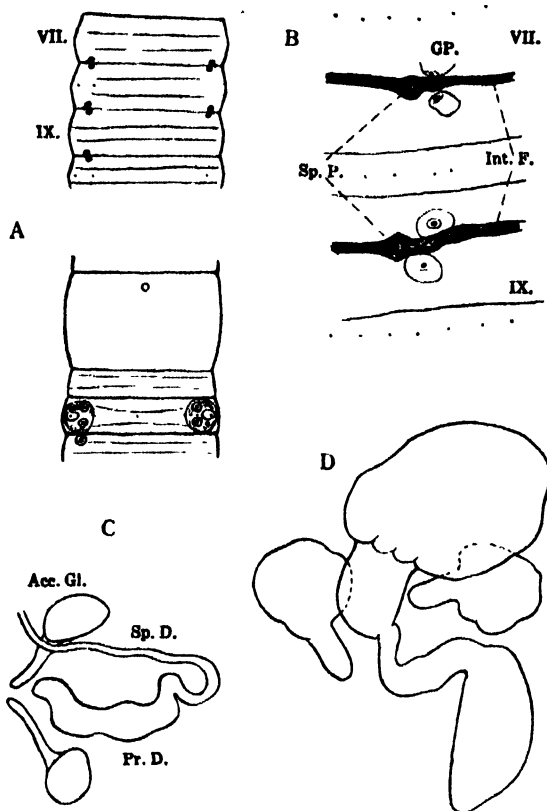


Fig. 2. *Pheretima masatacae*. A. Ventral view of VII-XIX. B. Spermathecal pore region, ca.  $\times 12$ , GP. genital papillae; Int. F. intersegmental furrows; Sp. P. spermathecal pores. C. Posterior male organs, Pr. D. prostatic duct; Sp. D. sperm-duct. D. Spermatheca and accessory glands corresponding to external genital papillae, ca.  $\times 12$ .



description of the present species is incomplete. Externally, he described only on the body size and situation of the genital papillae. We can only interpret from his descriptions not only on the present species, but also on *P. nipponica* that, it is provided with the usual external characters as in the latter species or in most species of the genus. On situation of the genital papillae, he described (externally) "the only point that may be specially mentioned is the existence of copulatory papillae on the same segments as those which bear the orifices of the spermathecae, viz. Nos. VIII and IX" and (internally) "There are four of these glands on each side. . . . Two open in the neighbourhood of each spermatheca". From the foregoing statement, I interpret that two copulatory papillae exist just close to each spermathecal orifice, as those in the present specimens. Internally, the important characters which were, at least, described in the original paper are approximately identical with those of the present specimens. In the foot-note of *P. communissima*, GORO and HATAI ('99) cited the present species as one of the species having the complicated caeca with several secondary caeca. But, it must be some mistake. CHEN's *Ph. ultoria* perhaps stands close to the present species; the character of the spermathecae is, according to him, closely resembling each other, but, against his interpretation, not in genital papillae and, rather, the aspect of the male pore segment resembles each other.

The geographical situation of this new locality of the present species seems to endorse to some extent HATAI's saying that a majority of the earthworms examined by European authorities were the forms which are mostly found in the south of Japan (1931, p. 401).

#### Appendix to *Ph. masatacae* (BEDDARD)

Three clitellate specimens were also collected from Chin-do, a small island, about 42 km. distant from Mokpo, in March, 1935. These are quite identical in all characters with the specimens of Quelpart Island, except the inconstant existence of genital papillae on 9/10 and the presence of quite vestigial prostatic glandular portion on one side of a specimen. Judging from that they are clitellate in March, the present species is perhaps a hibernated form.

#### *Pheretima kanrazana*, n. sp.

##### var. *typica*, n. var.

Mt. Kanra, about 1,000 M. in altitude, near Shiitakegoya, one clitellate and three juvenile specimens.

*Description :*

*External characteristics :* Length 92 mm, greatest diameter 4.5 mm, number of segments 90. Colour (in formalin), light brown dorsally, pale ventrally, clitellum light chocolate. Prostomium, epilobous, ca. 2/3. First dorsal pore in 12/13.

Setae beginning on II and small; no marked difference in size and interval throughout the body, but ventral ones may be partly slightly larger than the dorsal. Both ventral and dorsal breaks very slight if present. Setal number as follows: 34/III, 36/IV, 53/VIII, 56/XII, 56/XX, 19 (VI), 21 (VII), 22 (VIII) between spermathecal pores, 15 between male pores.

Clitellum entire, in XIV–XVI, without setae.

Male pores ventrolaterally on the setal line of XVIII, about 1/3 of the circumference apart; each on a very small circular papilla, surrounded by a few circumferential furrows. The male marking as a whole slightly elevated but less medially, and apparently slightly glandulated. Just medial to the male marking, are found three circular small genital papillae with central depression rather sunken into the ground and arranged to straightly antero-posteriorly, two presetal and one postsetal (Fig. 3. A.).

Female pore single, on XIV midventrally.

Spermathecal pores, two pairs in 6/7 and 7/8, about 1/3 of the

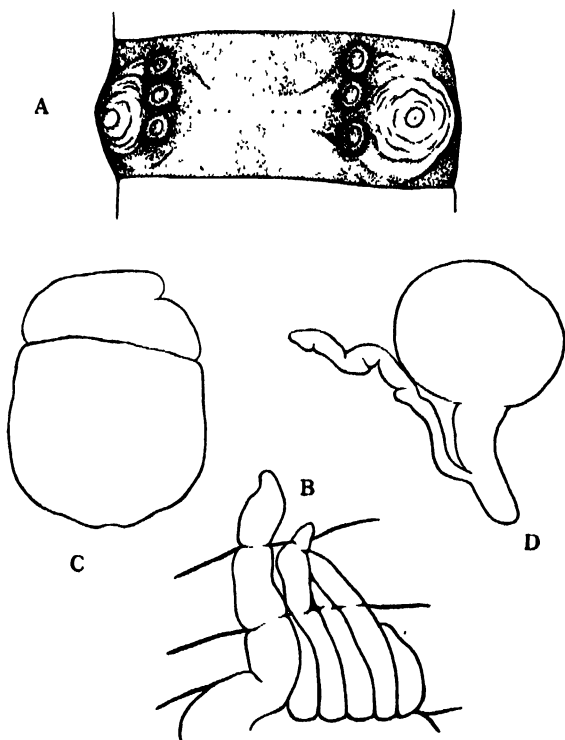


Fig. 3. *Pheretima kanrazana* var. *typica*, n. sp., n. var. A. Ventral view of XVIII, ca.  $\times 12$ . B. Caecum with secondary caeca, ca.  $\times 12$ . C. Posterior view of seminal vesicle, ca.  $\times 12$ . D. Spermatheca, ca.  $\times 12$ .

circumference ventrally apart; each on a minute tubercle sunken into the intersegmental furrow. No genital papillae found near this region.

*Internal anatomy:*

Any septa specially not thickened, 5/6 and 6/7 slightly thickened, 7/8, 10/11, and 11/12 thin, 8/9 ventrally traceable, 9/10 absent.

Nephridial tufts moderately thick, in V and VI.

Gizzard moderate in size, bell-shaped. Intestine beginning to swell in XV. Intestinal caeca small, originating in XXVII; each consisting of six secondary caeca, of which the dorsalmost is longest, extending as far anteriorly as XXIV, and with distinct septal constrictions, the more ventral ones become gradually shorter and smaller (Fig. 3. B.).

Hearts, four pairs in X–XIII, small in calibre; first pair smaller than the rest. Paired lymph glands found along dorsal vessel behind 15/16 caudalwards.

Testis sacs, two pairs in X and XI, small; anterior pair oval in shape, ventromedially connected with each other forming a V-shape, posterior pair fused with its fellow forming a transverse sac. Both sacs are in contact with each other. Seminal vesicles, two pairs in XI and XII, small, oval in shape, weakly granular on surface; each with a distinct large smooth dorsal lobe (Fig. 3. C.). Testes and funnels usual in position. Sperm-ducts on each side meeting in XIII. Pseudovesicles, one pair, moderate in size, attached to the posterior face of 12/13.

Ovaries moderate in size, usual in position.

Prostate gland moderate in size, in XVII–XX, consisting of about three main lobes; duct rather thin but muscular and shining on surface, looped in a hair-pin-shape with nearly equal thickness except the slightly thinner entalmost. Close and medially to the prostate, three small stalked accessory glands found corresponding to the external genital papillae.

Spermathecae in VII and VIII, small; ampulla round, about 1.3 mm or so in diameter; duct slightly longer than the ampulla, moderate in thickness, distinctly marked off from the latter; diverticulum slightly longer than, or nearly equal to, the main portion, its ental half moderately zig-zagly coiled, the ectal with nearly equal thickness to the former straight or simply twisted (Fig. 3. D.).

*Remarks:*

The present species is easily distinguishable from the other members of the genus having the complicated caecum which consists of a few or several secondary caeca, viz. *Ph. irregularis*, *Ph. agrestis*, *Ph. glandularis*, *Ph. levis*, *Ph. vesiculata*, *Ph. communissima*, *Ph. sieboldi*, *Ph. vittata*, *Ph.*

*schizopora*, *Ph. hilgendorfi*, *Ph. yunoshimensis*, *Ph. tappensis*, and *Ph. kikuchii*.

var. *incretata*, n. var.

Mt. Kanra, same spot where var. *typica* was collected, one clitellate (appears to be regenerated), two a clitellate, and one juvenile specimens.

**Description :**

**External characteristics :** Length ca. 80 mm, greatest diameter 4.5 mm, number of segments ca. 82. Anteroposterior segments to the clitellum with three annuli. Colour resembles that of var. *typica*. Prostomium, epilobous, ca. 1/2.

Dorsal pore in 12/13 indistinct and non-functional; the next distinct and first functional.

Setae beginning on II; those of X-XIII rather delicate, the rest small; no marked difference in size and also in interval between ventral and dorsal ones. Both ventral and dorsal breaks very slight,  $aa=1.1-1.4$  ab,  $zz=1.1-1.6$  zy. Setal number as follows: 30/III, 48/VI, 51/VIII, 50/XII, 52/XVII, 49/XX, 17 (VI), 18 (VII), 18 (VIII) between spermathecal pores, 14 between male pores.

Male pores ventrolaterally on the setal line of XVIII, about 3/8 of the circumference apart; each on a tiny tubercle placed on lateral part of large, oval, apparently glandulated, slightly elevated genital area which is anteroposteriorly occupying a whole segment and transversely about ten setal wide or about 2.3 mm. Genital areas on both sides are rather close remaining midventrally a shorter distance than the transverse length of each area, so most of the male pore setae are planted on these areas, but are not modified in shape and also in size. On middle part of the area, are found six or seven small circular genital papillae with slight central depression rather sunken into the ground, five crowding together presetally and one or two postsetally (when two papillae present they are arranged transversely) (Fig. 4. A.).

Female pore single, on XIV midventrally.

Spermathecal pores, two pairs in 6/7 and 7/8. Situation and structure of these markings are similar to those of var. *typica*. No genital papillae found near this region.

**Internal anatomy :**

Any septa specially not thickened, 5/6-7/8 slightly thickened, 8/9 ventrally present, shoved back to the posterior side of gizzard, 9/10 absent, 10/11-13/14 thin; 11/12-13/14 are much displaced posteriorly by pushing

of the large seminal vesicles and testis sacs.

Nephridial tufts moderately thick, in V and VI.

Gizzard small, bell-shaped, lying in IX-X. Intestine beginning to swell in XV. Intestinal caeca originating in XXVII, small but complicated; each consisting of three secondary caeca, of which the dorsalmost is slightly longer and larger than the rest and reaches to about XXV, the more ventral ones are nearly equal in size and in shape to each other and reach to a part of XXV. In one acitellate specimen, left side caecum

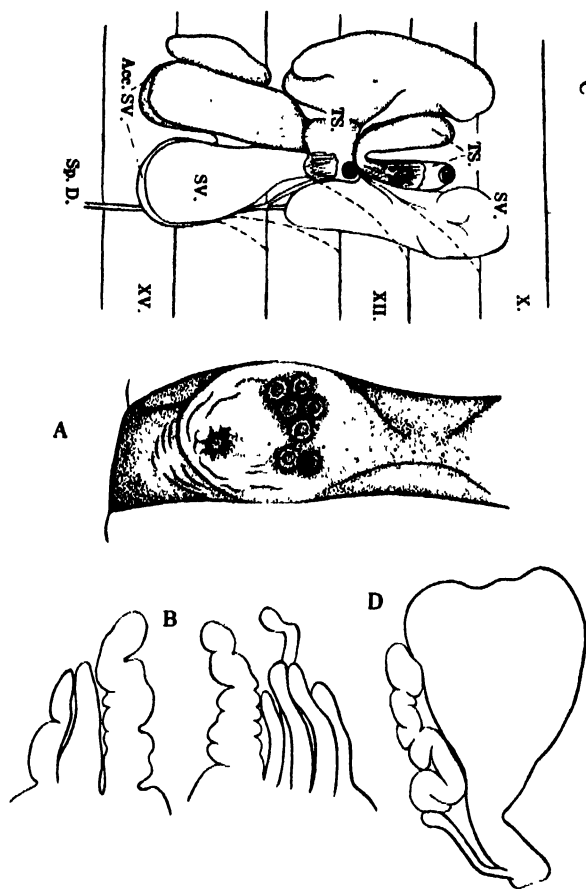


Fig. 4. *Pheretima kanrazana* var. *increta*, n. var. A. Ventrolateral view of XVIII, ca.  $\times 12$ . B. Intestinal caeca taken from an acitellate specimen, ca.  $\times 12$ . C. Anterior male organs (dotted lines indicate the displacement of each septum); SV. seminal vesicles; TS. testis sacs; Sp. D. sperm-duct; Acc. SV. accessory seminal vesicles. D. Spermatheca, ca.  $\times 12$ .

consists of three secondary caeca, of which the dorsalmost is largest, extending to XXV, and with several serriformed outgrowths on both dorsal and ventral margins, the right side one consists of six secondary caeca, of which the dorsalmost is largest and with outgrowths, the second smallest, and the third longest but much thinner than the first (Fig. 4. B.).

Hearts, four pairs in X-XIII, small in calibre; first pair much smaller than the rest or may be said to be vestigial (but, not vestigial in a clitellate specimen). Paired lymph glands found along dorsal vessel behind 15/16 caudalwards.

Testis sacs, two pairs, conspicuously large, especially the anterior pair. Anterior pair placed on the anterior face of 10/11, posterior pair in contact with the former. But, actually both pairs are much displaced posteriorly, the anterior pair situated in  $1/2$  XI-XII  $1/3$  and the posterior pair in  $2/3$  XII-XIII  $1/2$ . Each of the anterior pair cylindrical in shape, ventromedially scarcely connected with its fellow at their posteriormost part forming an elongate U-shape, posterior pair much shorter but with equal width to the anterior, fused with each other forming a quadrate sac. Seminal vesicles, two pairs, very large, voluminous, meeting middorsally one another, rather smooth on surface; anterior pair cylindrical in shape, larger than the posterior, occupying about three segments, from a small part of X-XIII  $1/4$ , each communicated with the corresponding testis sac at its middle ventral portion, with an indistinct primary ampulla which is projecting posterodorsally from its posterior margin; posterior pair elongate purse-shaped, more voluminous at its posteriormost, occupying about two segments, from  $1/2$  XIII-XV  $2/3$ , communicated with the corresponding testis sac at its anterior narrow end, with a large cylindrical primary ampulla which is similar in texture to the main vesicle and is projecting dorsally from the dorsal margin of the vesicle. Testes moderate in size, funnels large; both organs are usual in situation and structure. Sperm-ducts on each side meeting in posterior part of XIII. Pseudo-vesicles, one pair, relatively large, attached to the posterior face of 12/13 (ventrolaterally to the accessory seminal vesicles). Ventrally posteriormost margins of the two posteriorly displaced septa 12/13 and 13/14 appear to be fused forming two large but rather sheet-like chambers, so-called accessory seminal vesicles; each containing a little whitish mass of seminal products, situated beneath the voluminous part of each vesicle of the second pair, as like a neck-rest of them. In an a clitellate specimen, the following structures of the anterior male organs were observed. Testis sacs, two pairs, large, both nearly equal in size and in shape, similarly

V-shaped but the posterior pair slightly broader and lower, posteriorly displaced but not marked as in the clitellate specimen, the anterior pair situated in  $1/3$  XI–XII  $2/3$  and the posterior in XII. Seminal vesicles, two pairs, moderately large, both oval in shape and nearly equal in size; anterior pair situated in  $1/4$  XI–XII  $1/2$ , posterior pair in  $1/2$  XII–XIII  $1/2$ ; the latter pair with distinct dorsal lobe, that of the former indistinct. Septa 10/11–12/13 posteriorly displaced (Fig. 4. C.).

Ovaries moderate in size; by the posteriorward displacement of septum 12/13 situated at the posterior part of XIII, or just anteriorly to the oviduct funnels which are normally placed.

Prostates are similar to those of var. *typica*. Medially to prostate, small accessory glands with rather thick stalk found corresponding to the external genital papillae.

Spermathecae in VII and VIII, small; ampulla is not constricted off or sharply differentiated from the duct which is very short but moderate in thickness, so the main portion as a whole somewhat resembles a funnel in shape. Anterior part of ampulla slightly flattened, weakly darkened and measured there about 2.3 mm. Diverticulum always slightly longer than the main portion if stretched, its ectal fourth slender or rather fine, nearly straight, the ental remaining a trifle thicker than the former and moderately zigzagly coiled on one plane (Fig. 4. D.).

*Remarks :*

This form was once considered as a species distinct from *Ph. kanrazana* for the quite difference of some important characters, such as aspect of male pore segment, anterior male organs, and shape of spermathecae. But now I think it is more advisable to place it in varietal rank of the latter species on account of their same habitat and several important characters which are identical with each other, viz. the body size, number of segments, position of the first dorsal pore, setal number, aspect of the spermathecal pore region, thinness of the septa, vascular system, structure of the posterior male organs, complicated caecum with secondary caeca; and moreover, since the minor differences between them, such as number of spermathecal setae, position of first dorsal pore, and degree of development of the secondary caeca may fall within the extent of variation of both forms if a larger number of specimens were examined respectively.

Number of genital papillae on the male pore area appears more or less to be constant, viz. in one clitellate (type) and two aclitellate specimens, 5(presetal)/2(postsetal) on left side and 5/1 on right side; 5/2 and 5/2; 5/2 and 5/1. Unusual displacement of the anterior male organs

which appears to be constant (?), may be a quite different character from var. *typica*. Judging from the structures of the juvenile or acitellate specimens, the posteriorward displacement of the anterior male organs, ovaries, and of the corresponding septa, and also the formation of the accessory seminal vesicles may be secondary.

### *Pheretima quelparta*, n. sp.

Saishū, Japanese garden, 24 clitellate, 6 acitellate, and 7 juvenile specimens.

Seikiho, hill side, one clitellate and one acitellate specimens.

Mt. Kanra, mainly at about 1,000 M and 1,800 M in altitude, 4 clitellate, one acitellate, and 4 juvenile specimens.

#### *Description :*

*External characteristics :* Length 115–160 mm, greatest diameter up to 7.5 mm, number of segments 110–127. All segments, except both anterior and posterior ends of the body, with three or four annuli. Colour, characteristic, greenish blue dorsally, light blue ventrally, clitellum russet. Prostomium epilobous, ca. 1/3–1/2.

First dorsal pore usually in 12/13; but, it is variable as follows :

12/13, functional .....	26 specimens
12/13, distinct but non-functional.....	5
11/12, non-functional pore-like marking .....	2

Setae beginning on II and, in general rather small; setal breaks slight, aa=1.1–1.6 ab, zz=1.6–2.7 zy; setae on II or III–IX and on the posterior end of the body enlarged, those of X–XIII smaller than the rest of the body; no marked difference in size and also in interval between the dorsal and ventral. Setal numbers of a few specimens picked up at random are indicated belows :

III	IV	VI	VII	VIII	XI	XII	XVII	XX	MP	SVI	SVII	SVIII
41	45	51	53	54	62	60	58	56	20	17	18	20
37	42	55	55	56	60	60	47	60	19	18	18	19
39	44	47	50	55	52	49	47	51	18	16	17	17
40	46	50	54	57	62	59	63	58	21	18	18	20

MP....male pore setae; SVI, etc....spermathecal setae

Male pore setae varied in 18–24, of these 2–4 setae are usually found on each male genital area and are slightly larger than the rest but similar in shape; spermathecal setae 16–19 (VI), 16–19 (VII), 17–21 (VIII).

Clitellum entire, in XIV–XVI, without setae; mostly slightly constricted.

Male pores situated ventrolaterally on the setal line of XVIII, about 1/3 of the circumference apart, in a shallow copulatory chamber, each



opening is represented as a lateral crescent-shaped slit placed on a watch-glass-like elevation and occupies anteroposteriorly a whole segment. Inner wall of the chamber slightly wrinkled and glandulated, on its medial wall is usually present a circular genital papilla with slight central depression. In the majority of the present specimens the chambers are everted. In such cases, the male genital area is considerably protruded forming a disc with marginal ridges, and is slightly smaller in the anteroposterior length than the normal case, occupying about  $4/5$  or less of the segment remaining small parts on both anterior and posterior sides; surface of the disc appears to be glandulated, and genital papilla externally visible on it (Fig. 5. A and B.).

Female pore single, on XIV midventrally.

Spermathecal pores, three pairs in intersegmental furrows of 6/7-8/9, about  $1/3$  of the circumference ventrally apart; each represented as a large transverse slit, about three or four setal wide, internally each slit is always accompanied with a characteristic saccular body which is formed by the complicated foldings of the circular muscle layer. In the majority of the present specimens the saccular body is everted and protruded out its most or small parts in the intersegmental furrow. When completely everted, it is ovoid, much folded, not softened, conspicuously large, transversely about four or five setal wide, and among those foldings is usually found a distinct transverse groove along the intersegmental furrow which corresponds to the spermathecal opening in this case (Fig. 5. C.).

No more genital markings found elsewhere.

#### *Internal anatomy:*

Septa 5/6-7/8 and 10/11-13/14 very much or much thickened, 8/9 and 9/10 absent.

Gizzard moderate in size, globular in shape. Intestine beginning to swell in XV. Intestinal caeca originating in XXVII, thick but not so long, extending as far anteriorly as a part of XXIII, each with several serriformed outgrowths on ventral margin only, and with septal constrictions.

Hearts, four pairs in X-XIII, rather small in calibre; first pair smaller than the rest. Paired lymph glands found along dorsal vessel behind caecal segment caudalwards.

Testis sacs moderate in size, two pairs in X and XI; anterior pair oval in shape, connected ventromedially with its fellow forming a low V-shape, posterior pair fused forming a somewhat rectangular sac ventrally. Seminal vesicles moderate in size, two pairs in XI and XII; both pairs

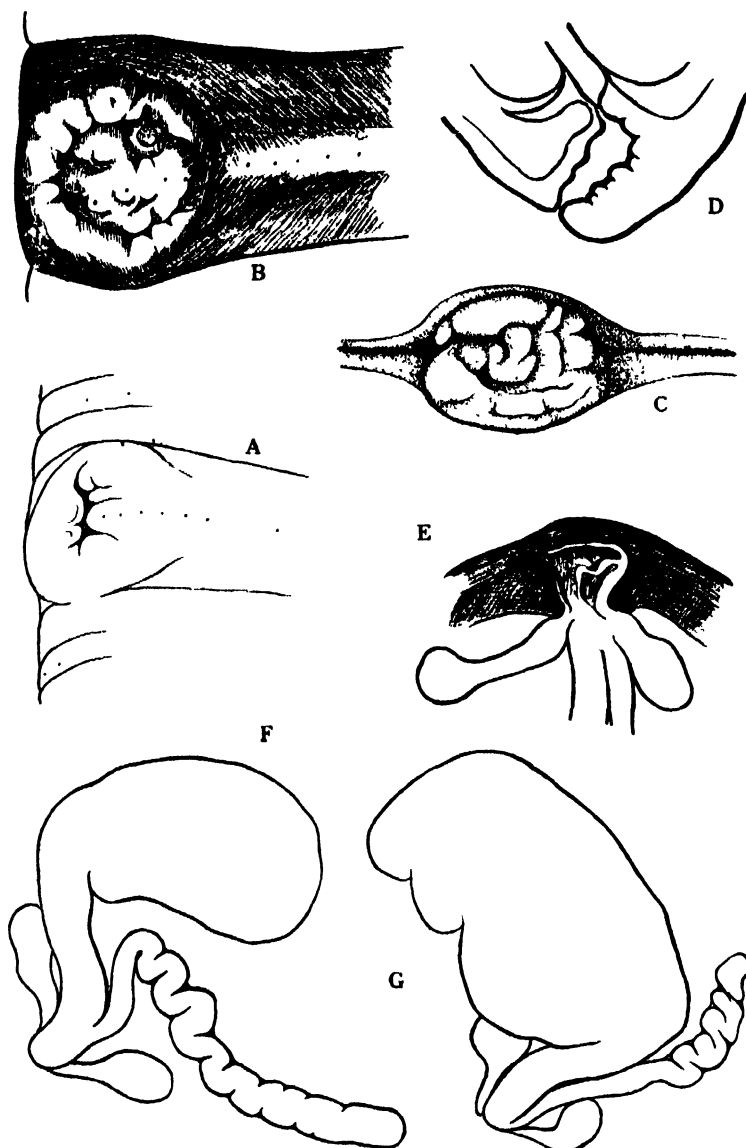


Fig. 5. *Pheretima quelparta*, n. sp. A & B. Ventral view of XVIII; A normal, ca.  $\times 8$ , B everted, ca.  $\times 11$ . C. Saccular body (completely everted), ca.  $\times 15$ . D. Transverse section of copulatory chamber, ca.  $\times 8$ . E. Transverse section of body wall through saccular body which is retracted, ca.  $\times 8$ . F & G. Spermathecae taken from separate specimen, ca.  $\times 8$ .

similarly circular in shape and the posterior pair slightly larger than the anterior; dorsal lobe of each vesicle large, sometimes irregularly subdivided into three or four small lobes; both vesicles and lobes are smooth on surface. Testes and funnels in usual position. Sperm-ducts on each side meeting in XIII. Pseudovesicles absent.

Ovaries moderate in size and usual in position.

Prostates moderate in size, in XVII-XIX, each consisting of about four main lobes; duct moderately thick and long, muscular and shining on surface, looped in a C- or hair-pin-shape, ectalwards gradually increasing its thickness in a small degree and finally with slightly decreased thickness entering into a small copulatory chamber. Near the ectal end of the duct usually found a circular small accessory gland with short stalk corresponding to the external genital papillae (Fig. 5. D.).

Spermathecae large, three pairs in VII-IX; ampulla large, ovoidal or elongated pear-shaped; duct moderate in length and thickness, slightly shorter than the ampulla, not sharply marked off from the latter, diverticulum either a little shorter or longer than the main portion, its ectal half nearly straight or simply twisted, the ental half zigzagly coiled on one plane and enclosed within a delicate sheath. Just close and antero-posteriorly to the ectal end of the spermathecal duct, are found two large accessory glands with long stalk which correspond to the saccular body (Fig. 5. E, F, and G).

*Remarks:*

The present species may be related to *Ph. tschiliensis* MICHAELSEN in character of spermathecae, but it differs from the latter not only in structure of the ectal end of the spermathecal duct, but also in the other many important characters. It is easily distinguishable from the other both Japanese and Korean species of the genus in characteristic colouration and saccular body.

The saccular body is formed by the complicated foldings of the circular muscle layer and is embedded within both circular and longitudinal muscle layers, and is associated with two large accessory glands. The stalked portion of the gland is embedded itself within the layers when the saccular body everted, and it is rather free laying its most part on the parietal wall when retracted.

The present species is the most common earthworm in this small volcanic island.

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# CHANGES OCCURRING IN THE EGG-LAYING ACTIVITY OF THE STRAWBERRY WEEVIL, *ANTHONOMUS BISIGNIFER* SCHENKLING, IN THE CASE OF A SOLAR ECLIPSE

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(With two figures)

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## INTRODUCTION

It is well known that the diurnal activity of the insect is in many cases not due to its own nature, but depends mainly upon the diurnal variation of climatic conditions. It is very interesting to observe the diurnal activity of insects in such a case as a solar eclipse, as the climatic conditions are variously changed at that time.

NEWPORT (1837) made some interesting observations on the Honey-bee and on a beetle, *Geotrupes stercorarius* L., in the case of the solar eclipse which occurred on the 15th of May in 1836. According to his investigation it is said that the sunshine diminished so remarkably in the course of the advance of the solar eclipse that the Honey-bees returned to their hives, and beetles, which were accustomed to fly in the evening, began to fly about, and then when the eclipse was over, the bees again came out from their hives and the beetles were not seen flying about.

In my previous paper we have recognized the fact that both the total radiant energy and the soil surface temperature affect to a large extent the activity of the egg-laying in the case of the Strawberry Weevil (KATÔ, 1937). Judging from the above fact it is interesting to observe the activity of the oviposition in the case of a solar eclipse, as the solar energy is greatly decreased and goes down even to zero at that time.

Quite recently, in the case of the solar eclipse which occurred on the 19th of June of 1936 in some part of Hokkaido, I had a chance to clarify the influence of the marked decrease of the solar radiation and of the change of other climatic factors upon the diurnal activity of the egg-laying of the said weevil.

## MATERIAL AND METHOD

Through the courtesy of the members of the Hokkaido Agricultural Experiment Station I was able to carry out fairly thoroughly the present investigation at the Kotoni Experimental Orchard near Sapporo which belongs to the same station (Fig. 1). In that orchard I used 308 strawberry plants for experiment and 116 plants for a supplementary experiment.



Fig. 1. The strawberry garden in the Kotoni Experimental Orchard of the Hokkaido Agricultural Experiment Station, where the present experiment was made (SHIZUO KATŌ photo.).

The experiment was carried out on the three days of the 18th, 19th and 20th of June and during that period I recorded the number of eggs laid every day at intervals of 100 minutes from sunrise to sunset. Thus I examined the strawberry garden nine times a day, viz. at 6.20, 8.00, 9.40, 11.20, 13.00, 14.40, 16.20, 18.00, and 19.40.

The solar eclipse began in Sapporo and its vicinity at the 14th hour and 8.6 min., reached its maximum phase at the 15th hour and 21.9 min. and finished at the 16th hour and 28 min. The climax phase of the solar eclipse was 0.96.

In this experiment the total radiant energy, the soil surface temperature, the air temperature measured at the height of 10 cm. from the

soil surface and the evaporation were all taken into consideration as the climatic factors controlling the egg-laying activity of the said weevil.

The total radiant energy was measured by both the ROBITSCH-Actinograph and the KIPP & ZONEN-Soralimeter, the temperature by the OTA's self recording thermometer and the evaporation by the recording evaporation gauge designed by myself (KATÔ, 1937).

The record of the total radiant energy obtained by the self-recording method was copied on COMMERCIAL paper made by the Oriental Photo Industrial Co. Ltd. and this latter was cut into pieces corresponding to the unit of 100 minutes. Thus the total radiant energy shown in every 100 minutes was indicated by the weight of each piece of the paper above alluded to. The temperature was represented by the mean value of the measurements calculated every 20 minutes and the evaporation was shown in mm. by the relative value (KATÔ, 1937).

#### RESULTS AND DISCUSSION

In Sapporo it rained from the 16th until the morning of the 17th of June, but was fine on the 18th, 19th and 20th, and thus I was able to carry out my investigation fairly successfully.

The 19th of June on which the solar eclipse occurred was rather fine, though some clouds were observed before and during the solar eclipse. Accordingly the climatic change which occurred during the time of the eclipse was said to be fairly remarkable.

The number of eggs laid and the record of the climatic factors obtained on the 19th of June was shown in Table 1 and in Figure 2. As can be

TABLE 1.

*The frequency distribution of the eggs laid and the variations of the climatic factors recorded at the 19th of June on which the solar eclipse was observed.*

Time-Interval	Egg-Number	Soil-Temp.	Air-Temp.	Total Rad. Energy	Evaporation	Egg-Number (20th day)
h. h.	(supplement)					
6.20-8.00	19 (—)	21.0°C	21.8°C	27.0 mg.	2.5 mm.	8
8.00-9.40	42 (13)	25.7	28.8	31.8	4.0	21
9.40-11.20	45 (22)	28.4	30.5	36.1	6.8	27
11.20-13.00	49 (24)	28.7	30.9	38.9	4.3	22
13.00-14.40	37 (19)	29.6	31.0	31.9	7.2	15
14.40-16.20	16 (8)	25.3	25.2	18.7	4.3	16
16.20-18.00	27 (14)	26.1	25.6	22.4	3.6	9
18.00-19.40	6 (—)	19.3	17.1	5.0	1.0	—



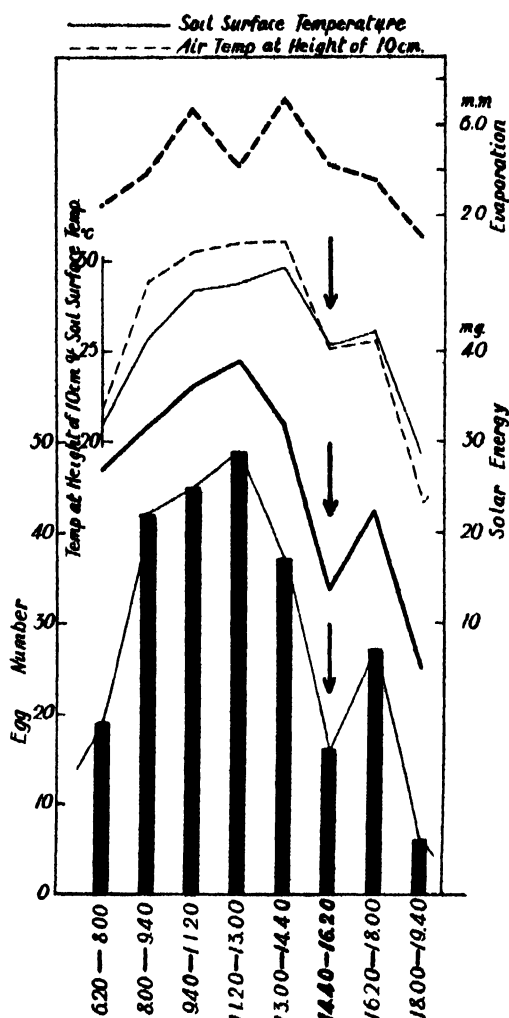


Fig. 2. Correlation figure showing the frequency histogram of the eggs laid and the variations of the climatic factors observed on the 19th of June, on which the solar eclipse was observed.

From Figure 2 it is evident that the evaporation is entirely independent of this phenomenon above mentioned.

The air temperature and the soil surface temperature decreased at the time of the solar eclipse which covered the period from the 14th hour and 40 min. to the 16th hour and 20 min., but this fall of the tempera-

seen from these, the egg-laying activity first increased markedly after the 8th hour and was most active in the period extending from the 11th hour and 20 min. to the 13th hour, and then it decreased gradually. But in the period extending from the 14th hour and 40 min. to the 16th hour and 20 min., viz. that of the solar eclipse, the number of eggs laid suddenly decreased, becoming less than half the number laid during the foregoing period, and when the period of the solar eclipse ended, the said activity again increased and the number of eggs laid was nearly double compared with the number of eggs laid in the period of the solar eclipse. No such phenomenon was observed on the next day, the 20th of June (Table 1), and thus it may be safely concluded that the decrease of the number of eggs laid during the solar eclipse was obviously due to the sudden climatic changes which occurred during the time of the same.

ture was not so remarkable as was the case of the decrease of the number of eggs laid, and moreover in spite of the fact that the number of eggs laid was very large in the time extending from 11th hour and 20 min. to the 13th hour, the maximum temperature was observed during the time extending from the 13th hour to the 14th hour and 40 min. Thus it may be recognized that neither the air temperature nor the soil surface temperature acts very strongly upon the activity of oviposition. But we can observe a perfect parallel phenomenon existing between the fluctuation of the solar radiant energy and that of the activity of egg-laying. Therefore in this case the radiant energy may be the most effective factor strongly controlling the activity of egg-laying.

In order to clarify the degree of correlation existing between the activity of egg-laying and the climatic factors, the coefficients of correlation were calculated from the data obtained on the 19th and the 20th of June by means of a direct method using the relative values of these characters above alluded to (Table 2).

TABLE 2.

*Coefficients of correlation calculated between the activity of the egg-laying and the climatic factors calculated from the data recorded at the 19th and 20th of June.*

Radiant Energy	Air-Temperature	Soil-Temperature	Evaporation
0.927 $\pm$ 0.025	0.847 $\pm$ 0.051	0.827 $\pm$ 0.057	0.383 $\pm$ 0.154

From the above we know that the total radiant energy controls the activity of egg-laying more markedly than the other factors do, the coefficients of correlation being 0.927. Concerning the evaporation the correlation was almost unnoticeable.

In conclusion we may say that the activity of egg-laying of the Strawberry Weevil was remarkably weakened during the period of the solar eclipse and recovered again when it was over. In this case it may be considered that this phenomenon was caused mainly by the sudden decrease of the solar radiant energy.

#### SUMMARY

1) In order to know how the diurnal activity of the egg-laying of the Strawberry Weevil is influenced by the climatic changes caused by a solar

eclipse, an experiment was conducted by myself at the Hokkaido Agricultural Experiment Station, situated near Sapporo, on the three days of the 18th, 19th and 20th of June of 1936. On the 19th day the solar eclipse was observed.

2) On the day of the solar eclipse the activity of egg-laying of the said weevil became rapidly active with the sunrise, but it decreased markedly with the advance of the solar eclipse and when the latter was over it became again active. This phenomenon may have been due to the change of the climate, which was caused mainly by the decrease of solar radiation.

3) From the coefficients of correlation which were calculated by a direct method, the existence of a close correlation between the activity of the egg-laying and the solar radiant energy was clarified.

The grade of the correlation was similar in the two cases of the soil surface temperature and of the air temperature measured at the height of 10 cm. above the soil. The grade of the correlation of the evaporation was so very low that it was scarcely noticeable.

#### ACKNOWLEDGMENT

I wish here to express my hearty thanks to Dr. SANJI HÔZAWA, Professor of Zoology of the Tôhoku Imperial University, who gave me kind guidance and instruction in the course of this investigation and also to Dr. ISAO MOTOMURA, Assist. Professor of the same, who gave me many valuable suggestions.

I am very grateful to Dr. SATORU KUWAYAMA, the Director of the Entomological Section of the Hokkaido Agricultural Experiment Station, for many valuable suggestions and much encouragement given me during the course of the present investigation. I am grateful also to Mr. SHIZUO KATÔ, a member of the same station, for his kind assistance. To Dr. SHOJI KONDO, Professor of Hygiene of the Tôhoku Imperial University, and to Dr. KOKI ABE, Assist. Professor of the same, I am much indebted for the meteorological instruments which I have used in the present experiment.

I desire also to express my great thanks to Dr. ZEN-EMON ÔNO, a member of the Entomological Department of the Hygienic Institute, Hsinking, Manchoukuo, and the former member of the Asamushi Marine Biological Station, who gave me special assistance, without which it would have been difficult for me to carry out the present experiment.

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## POSTSCRIPT

In the case of the solar eclipse which occurred on the 19th of June of 1936 in Japan, four biological investigations, as far as I know, were executed, by Dr. YOSHII in Sendai, by Dr. S. KUWAYAMA at Kotoni near Sapporo, Hokkaido, by Mr. S. MORI at Ômu in Kitami Province, Hokkaido, and by myself at Kotoni near Sapporo, Hokkaido.

The effect of a solar eclipse on green plants was dealt with by YOSHII (1936)<sup>1)</sup>.

According to the observation made by KUWAYAMA (1937)<sup>2)</sup>, the activity of the adult of the Rice Leaf-Beetle, *Lema oryzae* KUWAYAMA, was very much decreased in the course of the eclipse showing the similar condition of activity to that in the evening, i. e. about in the 18th hour and 30 min., except the mating activity.

In using trap MORI (1936)<sup>3)</sup> has mentioned that the number of flies (which specific name was not identified) captured was markedly diminished with the advance of the solar eclipse being influenced mainly by the decrease of the light intensity.

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<sup>1)</sup> YOSHII, Y. (1936): The Effect of a Solar Eclipse on Green Plants, Especially on Photosynthesis. (Japanese.) Ecological Review, Vol. 2, No. 4.

<sup>2)</sup> KUWAYAMA, S. (1937): An Observation on the Behavior of the Adult of *Lema oryzae* under the Eclipse of the Sun, June 19, 1936. (Japanese.) Botany and Zoology, Vol. 5, No. 1.

<sup>3)</sup> MORI, S. (1936): Solar Eclipse and Insect. (Japanese.) Scientific Knowledge, Vol. 16, No. 9.



# ON THE JAPANESE AQUATIC OLIGOCHAETA *CRIDRILUS MIYASHITAI*, N. SP.

By

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(With forty-three figures in text)

(Received February 1, 1937)

*Criodrilus bathybathe*s is initially described by STEPHENSON (1917), this description being based on some sexually immature specimens obtained from the bed of Lake Biwa at a depth of 180 feet.

Some years ago, some fixed and living specimens of an aquatic oligochaeta, which had been collected from a shallow ditch at Kômorî-Machi, a town in the Kyôto urban prefecture, were sent by Mr. Y. MIYASHITA to Prof. E. NOMURA. At first, NOMURA thought that, even though the species showed several similarities to *Criodrilus bathybathe*s STEPHENSON, it was probably a new one and to be distinguished from the latter, because of the difference in the depths of the habitats and of the presence of genital setae near the male pore of the former species.

In the spring of 1935, NOMURA suggested to NAGASE a study of the present species anatomically to ascertain whether it is the same species as *Criodrilus bathybathe*s STEPHENSON or not.

In order to collect specimens of this new species, NAGASE went to Kômorî-Machi in May, 1935, and succeeded in obtaining many of them.

Kômorî-Machi is surrounded by a range of hills on the north and west sides, and faces the River Yura on the south and east. The clear water from the hills, before reaching the Yura, flows between houses of the town through ditches, into which garbage and refuse from the houses are thrown. Such filthy places, however, are not a suitable habitat for the oligochaete worms under investigation, and they were found chiefly in stony places in ditches where there is a flow of clear water.

In a place, deeper than about 30 cm. and with a somewhat rapid flow of water, containing larger stones, the specimens collected were invariably smaller and the comparatively larger ones were found in a shallower place, containing smaller stones, near the edge of water with a somewhat slower flow. The same relation to stones was found even in places where there

was no water.

In the habitat, the worms usually protrude 2 or 3 cm. of the posterior portion of their bodies out of the mud and swing in the water, but lie on the mud without swinging when the water decreases in depth.

The worms were usually found scattered, and where they are congregated most densely, the swinging posterior portion may be counted as nearly one in every 1 cm. square. There were hardly any to be seen in a place in direct sunshine, but they were found in abundance in a place in shadow.

In June, 1936, we happened to hear from Mr. S. OHFUCHI that a specimen approximating to the present species had been found among a collection from Tsuruoka, a city in the Yamagata prefecture, and on going there, NAGASE again succeeded in obtaining many specimens of the same species.

The species under investigation was found in channels made for irrigating rice fields in the suburbs of the city of Tsuruoka. The depth of water in these channels was said to be from 50 cm. to 1 m. at ordinary times, but when NAGASE saw them, it was more than 2 m., owing to the increase of the water for irrigation in June. The bottoms of the channels were muddy, and covered with dead plants and stones. The sides of the channels were nearly vertical and soily, being grown over with plants indigenous to damp ground. In such conditions, the specimens collected from the bottom of the channel were, comparatively, smaller, and those found mingling with the roots of plants on the edge of the water generally larger.

We have, finally, reached the conclusion that, even though the species under discussion is closely allied to *Criodrilus bathybathe* STEPHENSON, it may not be identical with it. We, therefore, give in the following pages a detailed description of this species, for which we wish to propose the new name, *Criodrilus miyashitai*.

Before going further, we beg to express our sincere gratitude to Mr. KATSUHIRO OKADA and to Mr. IKUSÔ HAMAI for their helpful assistance, during the progress of our investigation. Our thanks are also due to Mr. YOSHINOBU MIYASHITA and to Mr. SUEKICHI TSUJI for their kindness shown at Kômorî-Machi, and to Mr. SHINRYO OHFUCHI and to Mr. KENJI NODA for their kindness shown at Tsuruoka, during the work of collecting the materials.

#### THE EXTERNAL FEATURES

When developed fully, the body has a pair of lateral swellings (Fig. 1),

the presence of which is the most prominent feature of *Criodrilus miyashitai*. Each lateral swelling is a quadrangular pyramid, its apex being located in Segment XIII, and its base extending from Segment XII to Segment XIV. The body is swelled out ventro-laterally, -- dorsally, at the level of the dorsal setae, and, ventrally, at the mid-line of the ventral body wall, so that, in cross sections through this region, it is crescent-shaped (Fig. 2). In the immature worms, the body length attaining nearly 10 cm., the indication of the lateral swelling may be discovered as a white papilla on either side of Segment XIII, at a level slightly towards the dorsal from the ventral setal line. *Criodrilus bathybathe*s at this developmental stage was probably studied by STEPHENSON.

Each full-grown, lateral swelling is divided into two papillae at its apex, an anterior and a posterior, remaining there as a vertical slit between them. When the worm is living, the papillae have an opening and closing movement. The slit between the papillae has a depth of 0.3 mm. or more.

The colour of the body is fluorescing reddish brown as a whole, but in the portion anterior to the lateral swellings is usually red, and is posteriorly shaded with light gray or yellow.

The length of a full-mature specimen reaches 25-30 cm., when perfectly narcotized, but nearly 20 cm., when fixed, and the width is nearly 4 mm. across Segment XIII, including the lateral swellings. The number of segments is about 250.

The prostomium is zygalobous. In longitudinal sections, it is always biannulate, consisting of a longer anterior and a shorter posterior annulus. Segments XII, XIII and XIV also consist of six or more annuli, and, when living, this region of the body appears much wrinkled.

In cross sections of the body, the portion anterior to the lateral swell-



Fig. 1. Photograph, showing fresh specimen of *Criodrilus miyashitai*. Natural size. A ventral view of sexually mature specimen, with its posterior portion lost, B dorsal view of sexually immature specimen.



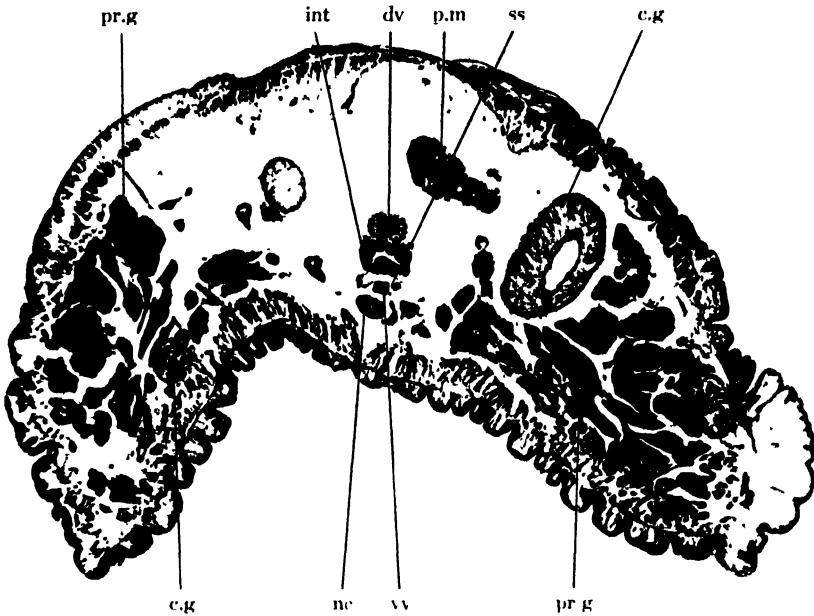


Fig. 2. Photomicrograph, showing crescent shape in cross section of body through Segment XIII.  $\times 30$ . *c.g* copulation gland, *dv* dorsal vessel, *int* intestine, *nc* ventral nerve cord, *p.m* peritoneal cell-mass, *pr.g* mass of prostate gland cells, *ss* septal sac, *vv* ventral vessel

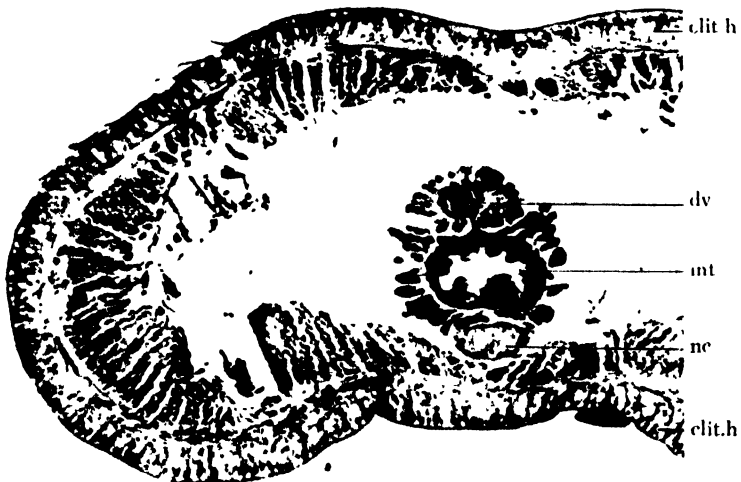


Fig. 3. Photomicrograph, showing kidney shape in cross section of body through Segment XV.  $\times 50$ . *clit.h* clitellar hypodermis, *dv* dorsal vessel, *int* intestine, *nc* ventral nerve cord.

ing is roundish, and the portion posterior to it is kidney-shaped with the concave side directed dorsally (Fig. 3), but the posterior portion of the body is elliptical, with the short axis directed vertically.

On either side of the body, the female pore opens just in front of the ventral seta-bundle in Segment XIV.

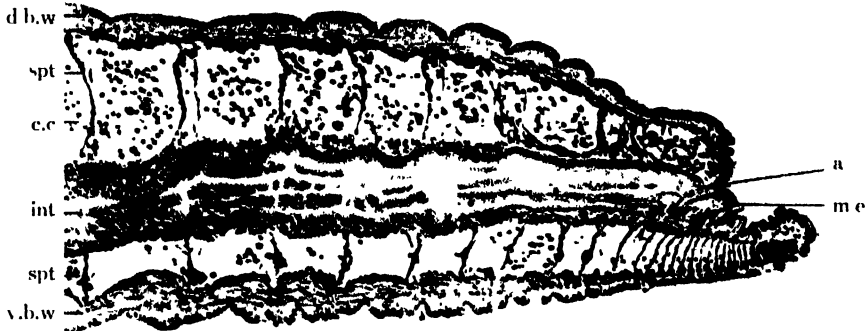


Fig. 4 Photomicrograph, showing position of anus in longitudinal section through median plane of posterior end of body.  $\times 50$  *a* anus, *c.c* coelomic corpuscles, *d.b.w* dorsal body wall, *int* intestine, *m.e* lateral lip of anus, *spt* septum, *v.b.w* ventral body wall.

The clitellar hypodermis begins shortly anterior to the female pore and is extended posteriorly to Segment XXXI or to Segment XXXIV, and the portion of the clitellar hypodermis posterior to Segment XVII is somewhat thickened in comparison with that anterior to it.

The anus opens on the dorsal side of the posterior end of the body.

#### THE BODY WALL, SEPTA AND COELOMIC CORPUSCLES

1) The body wall consists of a cuticular, a hypodermal, a circular muscle, a longitudinal muscle and a peritoneal layer in the order from the outside to the inside.

*a.* The cuticle. This layer is present throughout the body and is structureless. It measures nearly  $4\mu$  in thickness. At the two ends of the body, this layer is folded back inwards and is continued into the mouth and anal cavities, decreasing in thickness. The cuticular layer is specially thin over the sensory buds in the hypodermis and over the papillae of the lateral swellings.

*b.* The ordinary hypodermis. The hypodermis is a unicellularly arranged layer of columnar, gland, and supporting cells. This layer is thicker in the anterior region of the body, measuring nearly  $60\mu$ , but is

thinner in the posterior region, measuring only  $30\ \mu$ .

Each columnar cell contains cytoplasm which is thicker distally with a nucleus at the centre, and is generally  $20\ \mu$  or less in width, sometimes being much compressed.

Each gland cell is spherical or ellipsoidal, and is situated between the columnar cells. It contains thin cytoplasm and a nucleus. The shape and position of the nucleus is in every cell indeterminable. The proximal ends of the gland cells are often observed in the circular muscle layer.

The supporting cells are strongly compressed between the columnar cells and contain, respectively, a compact small nucleus, measuring  $5\ \mu$  in diameter.

Besides the cells above-mentioned, at the proximal base of the hypodermal cells, we often come across discoidal or conical cells and, — but not so often — the sensory buds, the cells of which show a bulbiform arrangement as drawn by VEJDOVSKY (1884) and by COLLIN (1888) in relation to *Criodrilus lacuum* HOFFM. The sensory buds are distributed more densely on the anterior than on the posterior region of the body. The distal end of the sensory bud projects slightly towards the cuticle, which is pierced by sensory hairs, and the proximal end reaches the circular muscle layer.

c. The clitellar hypodermis. As mentioned already, the clitellar hypodermis begins almost in front of the female pores on the ventral side of Segment XIV, but, on the dorsal side, it begins behind the ventral, starting at nearly the length of a segment or of a half segment. The clitellar hypodermis is considerably thicker than the ordinary hypodermis, measuring from  $160\ \mu$  to  $200\ \mu$ . In the portion of the clitellum anterior to Segment XVII and in the concaved dorsal portion of the clitellum, the hypodermis is thinner than that of the other portions of the clitellum. It is stated also by BENHAM (1887), in the case of *Criodrilus lacuum*, that the clitellar hypodermis is deeper at the sides than on the dorsal surface.

Three types of clitellar gland cells may be distinguished as follows:—

i) The club-shaped cells. The gland cells of this type measure  $30\text{--}40\ \mu$  in length, and, respectively, contain a nucleus, which lies at the proximal portion of the cell and is irregular in shape. Some of the cells are furnished with fine granules, which take the stain of haematoxylin, and some with large or fine granules, which are stainable with eosin. The position of the former cells in the hypodermis is indeterminable, but the latter are always arranged on the distal surface of the hypodermis.

ii) The elongated cells containing globules. These cells are comparatively slender, measuring  $75\ \mu$  or more in length and  $10\text{--}20\ \mu$  in width. The contained globules are nearly  $2\ \mu$  in diameter. The cells of this type never reach the inner face of the clitellar hypodermis.

iii) The elongated cells containing fine granules. The gland cells of this type are generally large, some reaching the inner face of the hypodermis. The width of these cells is  $30\text{--}10\ \mu$ . The nucleus is laid usually near the proximal base, but is sometimes attached to the lateral boundaries of the cell, and sometimes multinucleated.

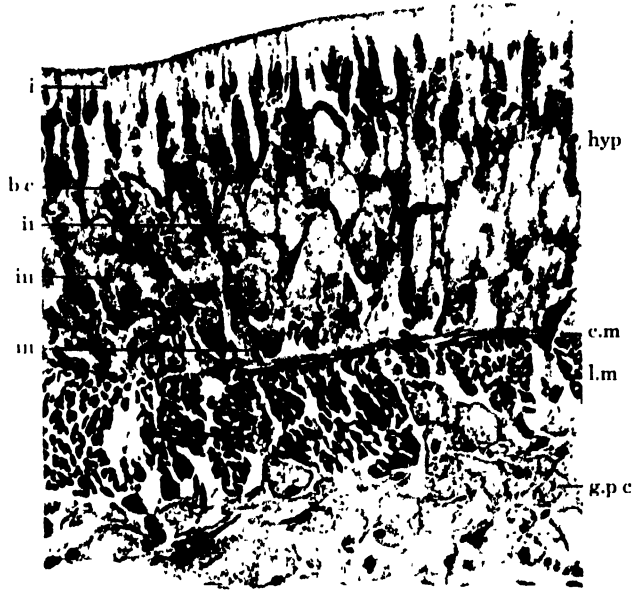


Fig. 5. Photomicrograph, showing structure of clitellum in cross section through dorsal body wall of Segment XVII.  $\times 200$ . *i* club-shaped cell, *u* elongated cell containing globules, *iii* elongated cell containing fine granules, *b.c* blood capillary, *c.m* circular muscle layer, *g.p.c* layer of glandular peritoneal cells, *hyp* hypodermis, *l.m* longitudinal muscle layer.

Blood vessels and muscle fibres with their nuclei are found between the gland cells. Moreover, a few cells, containing, respectively, a nucleus of  $5\ \mu \times 10\ \mu$ , are found between the hypodermis and the circular muscle layer. The nature of these cells is unknown.

*d.* The circular muscle layer. This layer is  $15\text{--}20\ \mu$  in thickness in the anterior region of the body, but decreases in thickness posteriorly and finally measures only  $7\ \mu$  near the anus.

*e.* The longitudinal muscle layer. This layer is considerably thick, especially in the clitellum, but very slightly developed in the prostomium. It is, in the clitellum,  $60\ \mu$  at the dorso-median and ventro-median walls,  $160\ \mu$  at the dorso-lateral and ventro-lateral walls, and  $230\ \mu$  on both sides of the body. In cross sections of the body, this layer is constructed

of bundles of irregularly or sometimes pinnately arranged longitudinal muscle fibres.

The lateral lines are present on both sides of the body, attached to the circular muscle layer with its distal base, being embedded in the longitudinal muscle layer.

*f.* The peritoneum. The peritoneal cells are generally irregular in shape, and are rich in granules and vacuoles, containing, respectively, an ellipsoidal nucleus. The granules are especially abundant in the peritoneal cells of the clitellar dorsal wall inside the dorsal setal lines. This portion of the peritoneum has a glandular appearance (Fig. 5 g.p.c) and attains nearly  $75\ \mu$  in thickness.

The peritoneal layer is transformed externally into a layer of connective tissue cells which are interwoven with the fibres of the circular and longitudinal muscles, and reach the hypodermis. The nuclei of the connective tissue cells between the longitudinal muscle fibres are almost compact and much elongated, measuring  $12\ \mu$  by  $3\ \mu$ . The connective tissue layer forms a membrane, which lies between the hypodermis and the circular muscle layer. This is especially thick beneath the clitellar hypodermis.

2) The septa. Septa II/III and III/IV are very thin and incomplete. A complete typical septum begins at IV/V. It is stated by STEPHENSON (1917), in reference to his specimens of *Criodrilus bathyathes*, that Septa V/VI XII/XIII are thick. In the anterior portion of the body, the septa are funnel-shaped round the alimentary canal, with their narrow ends directed posteriorly.

Each septum consists of three layers, viz. a thinner anterior peritoneal, a middle muscular, and a thicker posterior peritoneal.

3) The septal sac. This is a long sac, which lies on the ventral side of the alimentary canal in segments from VI to XIII, or rarely to XVI, but the beginning and ending of this sac were not definitely determinable. The sac is compressed dorso-ventrally and is extended laterally, being constricted by every septum existing in this region. The walls of the present sac consist of a thin unicellular layer of flattened cells. Its dorsal wall adheres to the ventral wall of the alimentary canal, and its ventral wall to the dorsal wall of the ventral vessel, along the median plane of the body (Fig. 2 ss). In the segments anterior to VIII, either lateral wall of the sac is attached by a longitudinal bundle of muscle fibres, the anterior continuation of which support the duct portions of the pharyngeal glands.

Inside the sac are found a number of coelomic corpuscles and a few cells resembling the chloragogen cells.

4) The coelomic corpuscles. These corpuscles are found more abundantly in the coelom of immature specimens than in that of mature ones, and in the posterior region of the body more than in the anterior. Each corpuscle is spherical in shape, measuring about  $10\mu$  in diameter, and contains eccentrically a compact nucleus, which is nearly  $3\mu$  in diameter.

#### THE SETAE AND THE SETA-BUNDLES

1) The ordinary setae. The setae are singly pointed and sigmoid, and are destitute of any ornamentation, except the nodulus which lies at two-thirds of the setal length from the proximal end. They are present from Segment II. Two setae are closely paired in a bundle.

Four bundles stand in the middle of each segment. The rectilinear distance between the dorsal and ventral setae is equal to that between the left and right ventral setae, when we take into consideration the points on the surface of the body, but the distance between the dorsal seta-bundles are always larger than that between the ventral, showing the proportion 1.3:1 in the segments anterior to XIII and 1.5:1 in the segments posterior to it.

In every segment, the dorsal setae (0.50–0.55 mm. in length) are always larger than the ventral setae (0.37–0.15 mm. in length), and the nodulus of the largest seta measures about  $40\mu$  in width.

In the region of a seta, the cuticular layer of the body wall is invaginated together with the hypodermis and forms a tube round the portion of each seta distal to the nodulus, closely investing it.

The seta-follicles of a bundle stand apart distally, but they closely adhere proximally, forming there a fundus of the seta-follicle, and being

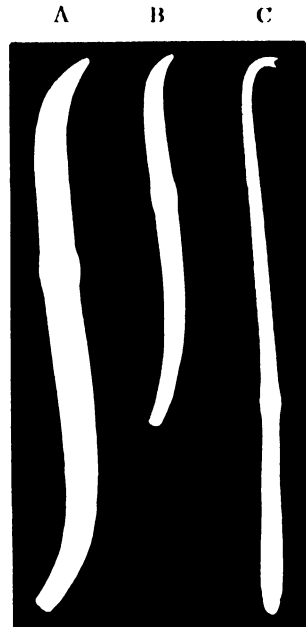


Fig. 6. Drawing, showing contour of setae  $\times 200$ . *A* largest dorsal seta from Segment IV, *B* seta from last segment, *C* genital seta.

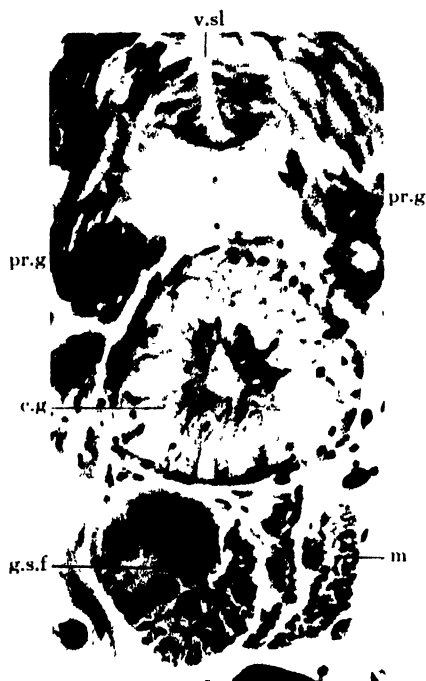


Fig. 7. Photomicrograph, showing relation between genital setae and copulation gland in cross section, from longitudinal section of sexually mature specimen.  $\times 200$ . *c.g* copulation gland, *g.s.f* genital setae follicle, *m* parieto-vaginal muscle, *pr.g* mass of prostate gland cells, *v.sl* vertical slit between papillae at apex of lateral swelling.

the lateral swellings are not yet formed, the same relation between the genital setae and the copulation gland is clearly observable (Fig. 8).

Each genital seta is slender, and measures 0.50-0.57 mm. in length and  $13\mu$  in width. An inconspicuous smooth nodulus lies at one-third of the setal length from the proximal end. The most distal portion of the genital seta is somewhat suddenly twisted and terminates at a narrowly bifurcate end (Fig. 6).

Each genital seta-bundle consists usually of four setae, sometimes of two or of six (Fig. 7), the distal ends being gathered towards the opening of the copulation gland.

The follicle of a genital seta is elongated and pear-shaped, with its

covered by peritoneal cells exteriorly. The nuclei of the seta-follicle are large, measuring nearly  $16\mu$  by  $17\mu$ , and contain, respectively, a large nucleolus.

The fibres of the parieto-vaginal muscle are better developed in the longitudinal direction than in the transversal. The retractor muscle fibres begin at the nodulus of each seta, and connect the dorsal and ventral seta-bundles of a corresponding segment, forming a thick bundle of muscle fibres on either side of the body.

In Segment XIII, the ordinary ventral setae are non-existent.

2) The genital setae. The genital setae are present, on either side of Segment XIII or the middle segment of the lateral swelling of a sexually mature specimen, in the vertical slit between the papillae just inside the opening of the copulation gland (Figs. 7 and 35). Even in an immature specimen, in which



Fig 8. Photomicrograph, showing relation of genital setae to other parts of body in cross section through Segment XIII of sexually immature specimen.  $\times 100$ . *c.g* rudimentary copulation gland, *f* fusiform cell or rudimentary prostate gland cell, *g.s.f* genital setae follicle, *v.b.w* ventral body wall

proximal end thicker than the distal. Each cell of the follicle, which surrounds the seta directly, contains a small nucleus, but the cells which are scattered round the follicle contain, respectively, a large nucleus furnished with a large nucleolus.

The parieto-vaginal muscle fibres are found connecting the follicle fundus of the genital seta-bundle and the musculature of the body walls of the lateral swelling. The retractor muscle fibres originate at the nodulus of each genital seta on the ventral side of Segment XIII and end at the nodulus of each ordinary seta on the corresponding dorsal side.

#### THE ALIMENTARY TRACT

##### 1) The mouth and the buccal cavity.

The mouth opens exteriorly at the beginning of Segment 1, and is situated ventro-posteriorly to the prostomium. It is continued directly into the buccal cavity, which lies confined in Segment 1. This cavity is flattened dorso-ventrally, and both lateral margins of its posterior part are lifted



Fig. 9. Photomicrograph, showing contour of genital setae.  $\times 200$ . *c.g* portion of copulation gland, *g.s* genital setae, *g.s.f* genital setae follicle, *v.sl* portion of vertical slit between papillae of lateral swelling.



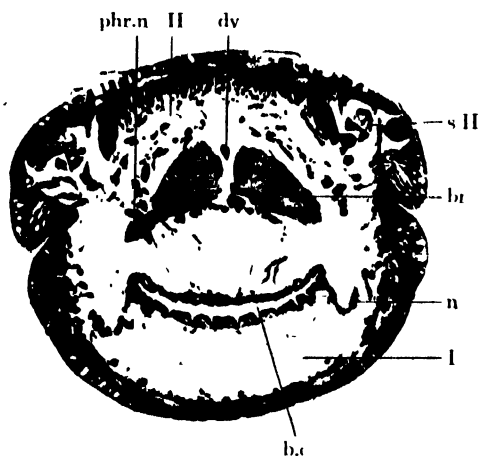


Fig. 10. Photomicrograph, showing contour of buccal cavity and of train in cross section of body.  $\times 50$ . *b.c.* buccal cavity, *br* brain, *dv* dorsal vessel, *n* branch of peri-pharyngeal nerve, *s.II* seta-bundle in Segment II, *I* and *II* Segments I and II.

dorso-laterally (Fig. 10).

The body walls are turned back into the buccal cavity through the mouth, so that the five layers of the latter are the direct continuations of those of the former: the innermost cuticle is very thin; the hypodermis is entirely destitute of gland cells; the muscle layers and the peritoneum are not well developed.

Internally, the ventral wall of the buccal cavity is rich in longitudinal foldings.

2) The pharynx. This is the direct posterior continuation of the buccal cavity, and lies in Segments II–1/2IV. When viewed from the dorsal side, it is nearly fusiform, and is suspended by muscle fibres (Fig. 11) extending antero-ventrally from the dorsal body wall just in front of Septum V/VI.

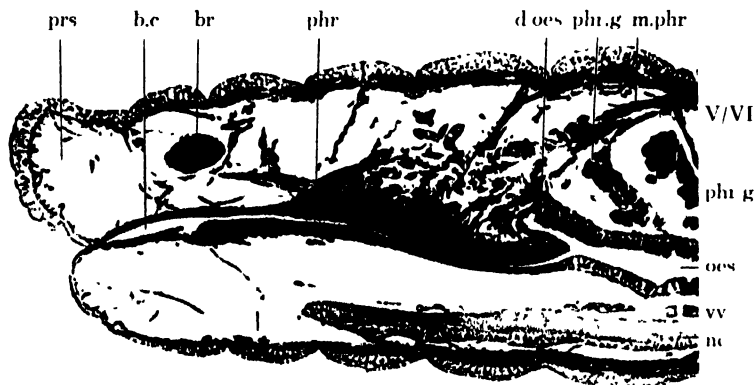



Fig. 11 Photomicrograph, showing pharynx in condition of eversion of buccal cavity and adjacent organs in sagittal section of immature specimen.  $\times 50$  *b.c* buccal cavity, *br* brain, *d.oes* diverticulum of oesophagus, *m* muscle fibres supporting pharynx, *nc* ventral nerve cord, *oes* oesophagus, *phr* pharynx, *phr.g* mass of pharyngeal gland cells, *prs* prostomium, *vv* ventral vessel, *V/VI* Septum V/VI.

The inner cavity of the pharynx is flattened dorso-ventrally, being extended laterally, and shows, in cross sections, a -shape, with a number of dorsal, and two lateral, longitudinal diverticula (Fig. 12) due to the foldings of the dorsal wall.

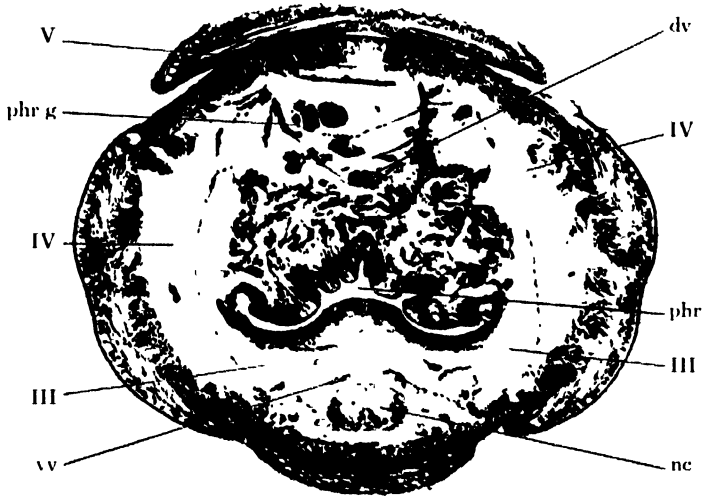


Fig. 12. Photomicrograph, showing pharynx and adjacent organs in cross section of body.  $\times 50$ . *dv* dorsal vessel, *nc* ventral nerve cord, *phr* pharynx, *phr g* mass of pharyngeal gland cells, *vv* ventral vessel, *III-V* Segments III-V.

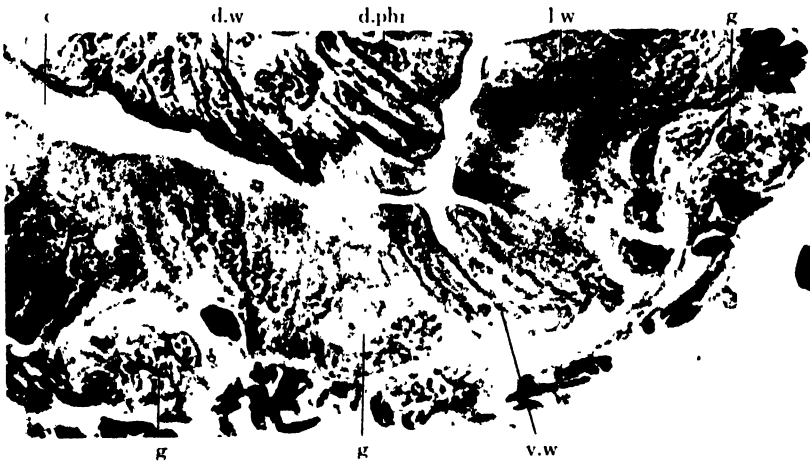


Fig. 13. Photomicrograph, showing peculiar gland cells at corner between ventral and lateral walls of pharynx in cross section of body through Segment III.  $\times 500$ . *c* pharyngeal cavity, *d.phr* duct of pharyngeal gland cell, *d.w* dorsal wall of pharynx, *g* peculiar gland cell, *lw* lateral wall of pharynx, *v.w* ventral wall of pharynx.

The inner pharyngeal endoderm consists of more or less glandular, columnar or fusiform cells, the distal face having a growth of cilia about

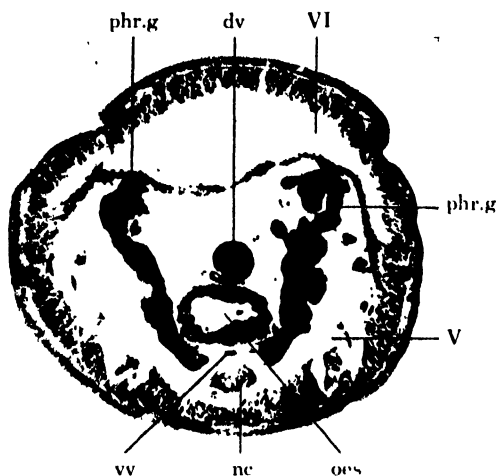


Fig. 14. Photomicrograph, showing pharyngeal glands and adjacent organs in cross section of young specimen.  $\times 50$  *dv* dorsal vessel, *phr g* pharyngeal gland, *nc* ventral nerve cord, *oes* oesophagus, *vv* ventral vessel, *V* and *VI* Segments V and VI.

a growth of cilia about  $7.5\mu$  in length. The cilia are more densely arranged in the dorsal wall than in the ventral. The nucleus is ellipsoidal, measuring nearly  $12.5\mu$  by  $6.5\mu$ , and is situated at the middle or in the proximal half of each cell.

The pharyngeal glands open to the dorsal endoderm, which is thicker than the ventral: the ducts of the pharyngeal gland take the stain of aniline blue and the endodermal cells the stain of orange G

in Mallory's connective tissue staining method, therefore both substances are easily distinguishable.

It is worthy of note, in connection with the pharynx, that on either side of it rows of peculiar gland cells are found near the corner between the ventral and lateral walls. Each of these gland cells is multi-nucleated and is rich in granules of different size (Fig. 13).

In the dorsal wall of the pharynx, the outer peritoneum is intermingled with muscle fibres and with blood vessels (Fig. 12), from which branch capillaries between the endodermal cells. The peritoneal and muscle layers are not well developed in the ventral wall, as compared with those in the dorsal.

3) The pharyngeal glands. The glandular masses are arranged in pairs (Fig. 14) in Segments IV–VIII. On either side of a corresponding segment, each mass of the gland cells is fixed in the coelom just in front of the posterior septum (Fig. 15) by muscle fibres starting from the dorsal body wall on a level with the inside of the dorsal setal line and by the fibres from the ventral body wall on a level with the inside of the ventral setal line.

Each pharyngeal gland cell is pear-shaped and contains a spherical nucleus, which is furnished with a nucleolus. Such cells are aggregated and form a cell-mass in the pharyngeal gland. The cells near the dorsal periphery of the cell-mass are directed with their narrow ends in the dorsal, and those near the ventral periphery are directed in the ventral direction. From these narrow ends, the narrow ducts start. These ducts form a bundle on either side of the body, being surrounded by a peritoneal connective tissue. The duct bundle proceeds ventrally at first and

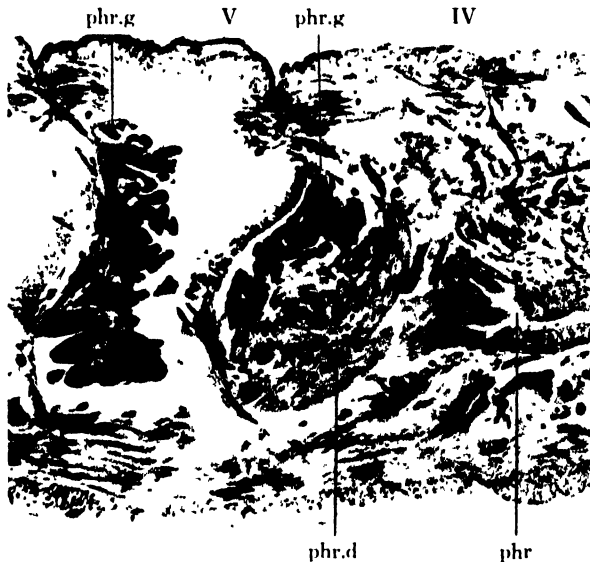


Fig 15. Photomicrograph, showing pharyngeal glands and adjacent organs in sagittal section of body.  $\times 50$ . *phr* pharynx, *phr.d* bundle of ducts of pharyngeal gland, *phr.g* pharyngeal gland, IV and V Segments IV and V.

then turns in the anterior direction. The duct bundles of a segment thus proceed into the next segment and are united with those of the latter segment, successively, on the ventro-lateral side of the alimentary tract and reach as far as the coelom of Segment IV. Then, the bundles are directed antero-dorsally and disperse themselves near the dorsal wall of the pharynx and, finally, open into it, separately (Fig. 13) or still forming bundles.

In the masses of the glands, the blood vessels are complicated between the gland cells; especially, those in Segments VII and VIII are traversed by the respective heart.

Further, amoebocytes are seen attached to the outer surface of each

gland, sometimes between the ducts or between the gland cells. Each amoebocyte is usually elongated fusiform, but is sometimes irregularly shaped. It contains a compact nucleus, and abundant granules, which take the stain of fuchsin. Similar cells are often found even in inter-spaces between the muscle fibres of the body wall.

4) The oesophagus. This portion of the alimentary tract is easily distinguishable from the pharynx by the absence of muscle fibres from the body wall, of longitudinal diverticula, and of ducts of the pharyngeal glands, and also by the dorso-ventrally flattened, elliptical outline of the oesophagus in cross sections.

The oesophagus begins at the middle of Segment IV with an oesophageal diverticulum, which is a dorso-anteriorly directed bulge from the

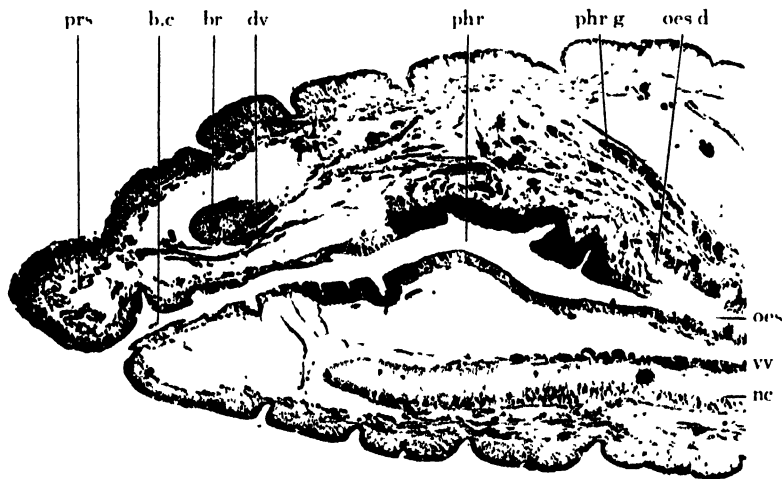


Fig. 16. Photomicrograph, showing anterior portion of alimentary canal and adjacent organs in median sagittal section of body.  $\times 50$ . *b.c* buccal cavity, *br* brain, *dv* dorsal vessel, *nc* ventral nerve cord, *oes* oesophagus, *oes.d* oesophageal diverticulum, *phr* pharynx, *phr.g* pharyngeal gland, *prs* prostomium, *vv* ventral vessel.

dorsal wall of the oesophagus (Fig. 16). Accordingly, the cross sections through this portion reveal the double walls of the endoderm, as shown in Fig. 17. This oesophageal diverticulum appears to us to be homologous with the evagination illustrated by Szűts (1913) at the anterior portion of the alimentary tract in the case of *Criodrilus lacuum*.

A small number of chloragogen cells are attached to the dorsal wall of the oesophagus.

5) The intestine. It is very difficult to distinguish the intestine from the oesophagus. In the case of *Criodrilus bathybathe*, STEPHENSON (1917) states that the intestine begins in Segment XII, with the chloragogen cells, which slightly increase in number. In our material, also it is true that the chloragogen cells increase in number in Segment XI or XII, but this increase does not invariably occur and there are no internal peculiarities by which the oesophagus and the intestine may be distinguished.



Fig. 17. Photomicrograph, showing oesophageal diverticulum at anterior end of oesophagus and adjacent organs in cross section of body.  $\times 50$ . *dv* dorsal vessel, *nc* ventral nerve cord, *phr.g* pharyngeal gland, *w.al.c* walls of alimentary canal at junction between pharynx and oesophagus, *w.oes.d* walls of oesophageal diverticulum, *IV* and *V* Segments IV and V.

The walls of the intestine consist of five layers: viz. the layer of the endoderm, of the intestinal blood vessels, of the circular muscle, of the longitudinal muscle, and of the chloragogen cells, in the order of from inside to outside. The chloragogen cells are all arranged unicellularly round the intestine. Each chloragogen cell contains an ellipsoidal nucleus in the centre, a number of granules of equal size, which take the stain of fuchsin, and, sometimes, a large vacuole at the proximal half. The muscle layers are very slightly developed except in the neighbourhood of a septum where they are thickly developed.

The intestine is at first flattened dorso-ventrally, but, posteriorly, it

gradually acquires a circular shape with an increase in diameter, and in the middle portion of the body, it occupies nearly half the depth and one-third of the width of the corresponding segment (Fig. 18). Then the intestine narrows very slowly towards the posterior end and finally opens into the anus.

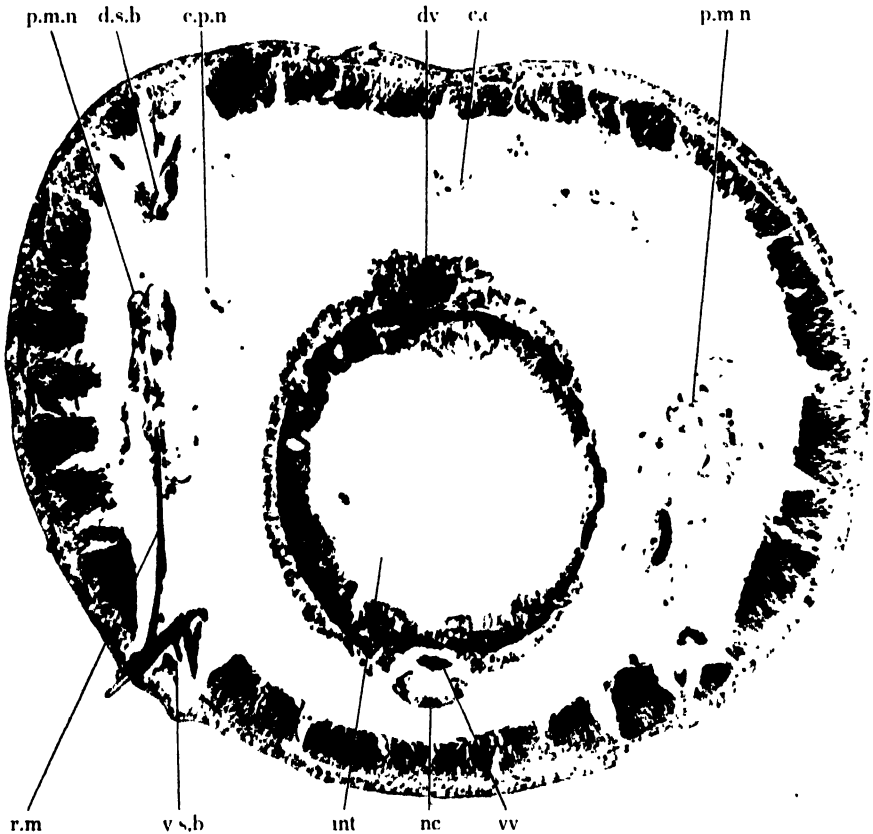


Fig. 18. Photomicrograph, showing intestine and adjacent organs in cross section of middle portion of body.  $\times 50$ . c.c coelomic corpuscle, c.p.n coiled portion of nephridium, d.s.b fundus of dorsal seta-bundle, dv dorsal vessel, int intestine, nc ventral nerve cord, p.m.n peritoneal cell-mass of nephridium, r.m retractor muscle, v.s.b ventral seta-bundle, vv ventral vessel.

On either side of the anal cavity, the lateral wall forms a swelling (Fig. 4), which may be homologous with the lateral lip described by STEPHENSON (1917) with reference to *Criodrilus bathyathes*.

## THE NERVOUS SYSTEM

The brain lies on the dorsal side of the buccal cavity between Segments I and II (Figs. 11 and 16), and is kept in position in the coelom by muscle fibres from the dorsal body wall. The brain is nearly rhomboidal as seen from the dorsal, with its longer diagonal parallel to the body axis, and measures 0.36 mm. or more in width and nearly 0.5 mm. in length. A shallow groove lies longitudinally on the dorsal surface of the brain along its median line. The posterior half of the brain is divided into two lateral portions by a median cleft. The anterior half of this cleft is a perforation, which admits the passage of the dorsal vessel (Figs. 10 and 19). Thus, the brain may be described as constituted of two

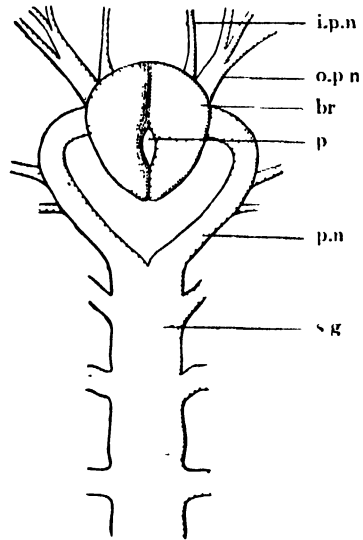


Fig. 19. Diagrammatic representation of brain and sub-pharyngeal ganglion.  $\times 50$ . *br* brain, *i.p.n* inner prostomial nerve, *o.p.n* outer prostomial nerve, *p* passage of dorsal vessel, *p.n* peri-pharyngeal nerve, *s.g* sub-pharyngeal ganglion.

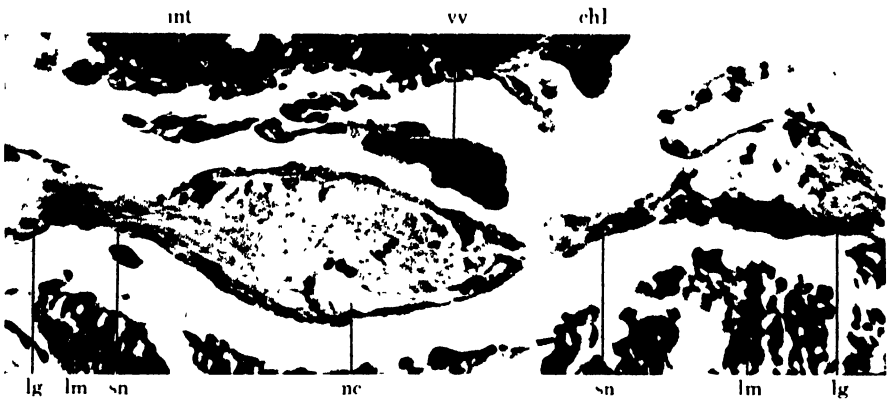


Fig. 20 Photomicrograph, showing relation between ventral nerve cord, lateral ganglia and adjacent organs in cross section of body through Segment XIV.  $\times 200$ . *chl* chloragogen cell, *int* ventral wall of intestine, *lg* lateral ganglion, *lm* longitudinal muscle layer of ventral body wall, *nc* ventral nerve cord, *sn* segmental nerve, *vv* ventral vessel.



lateral lobes. In cross sections, the brain, as a whole, is a plate 0.1 mm. in thickness, convexed dorsally, the ventral surface showing a smooth concavity.

From either antero-lateral margin of the brain extend the inner and outer lateral prostomial nerves, branches of which innervate the prostomium.

Each peri-pharyngeal nerve starts from the postero-lateral margin of the brain at a point posterior to the originating point of the lateral prostomial nerves. It runs postero-ventrally, giving off two branches anteriorly, and embracing the buccal cavity. Finally, both the left and right peri-pharyngeal nerves unite with the sub-pharyngeal ganglion, which lies in the posterior half of Segment II.

Each ventral ganglion occupies nearly the whole length of the corresponding segment.

Three pairs of segmental nerves start laterally from each ventral ganglion: an anterior pair lies just behind the anterior septum, a middle pair nearly in the middle of the segment, and a posterior pair at the middle of the distance between the middle pair of segmental nerves and the posterior septum. The middle pair is the largest of these nerves.

Each segmental nerve extends from the ventral ganglion, laterally at first, and soon enters a small lateral ganglion (Fig. 20). This nerve then turns in direction ventrally and enters the ventral body wall at the level of the inside of the ventral setal line.

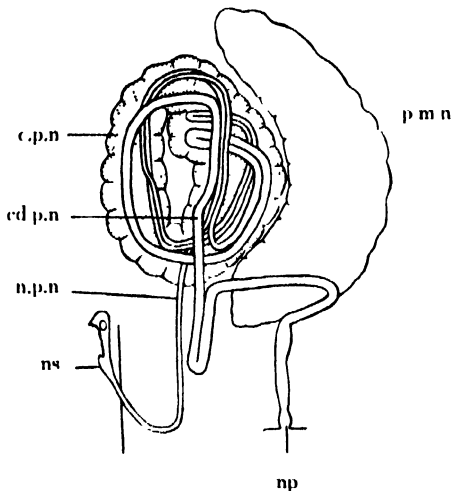


Fig. 21. Sketch of nephridium from compressed, immature specimen, showing its parts.  $\times 100$ . *c.p.n* coiled portion of nephridium, *cd.p.n* conduit portion of nephridium, *np* nephropore, *n.p.n* neck portion of nephridium, *ns* nephrostome, *p.m.n* peritoneal cell-mass of nephridium.

#### THE NEPHRIDIA

The nephridia occur in pairs in every segment posterior to XIV. Each nephridium consists of a nephrostome, a neck portion, a coiled portion, a peritoneal cell-mass, and a conduit portion with an ampulla (Fig. 21).

The nephrostome is attached

to the anterior face of a septum (Fig. 22) on a level with the inside of the ventral setal line. The structure of the nephrostome is nearly similar to that of *Criodrilus lacuum* described by VEJDovsky (1884) and to that of *Lumbricus terrestris* described by ROSEN (1911). In the species under investigation, the nephrostome consists also of an upper and a lower lip. The upper lip is discoidal, and consists of a number of central cells and a number of marginal cells, which surround the former. Either the central or the marginal cells are arranged unicellularly. The lower lip is considerably smaller than the upper, and the cells are very small, being arranged compactly. Both lips are ciliated internally.

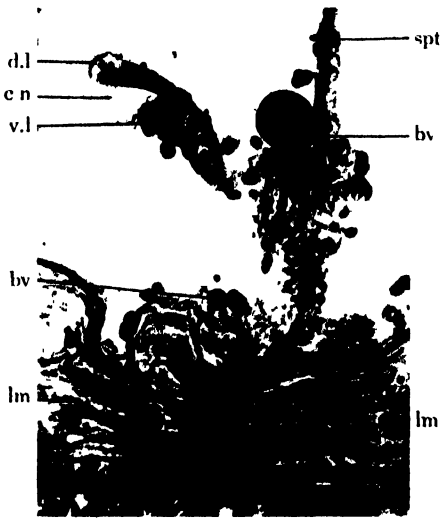


Fig. 22. Photomicrograph, showing nephrostome in sagittal section of body.  $\times 200$ . *bv* blood vessel, *c.n* cilia of nephrostome, *d.l* upper lip of nephrostome, *lm* longitudinal muscle layer of ventral body wall, *spt* septum, *v.l* lower lip of nephrostome

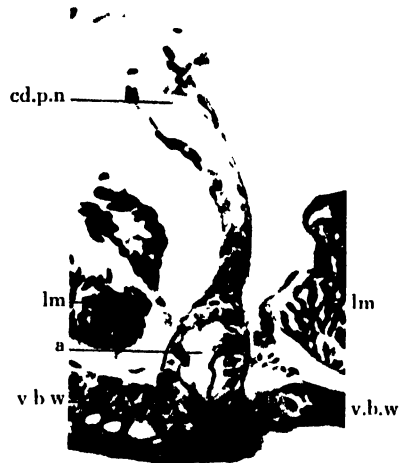


Fig. 23. Photomicrograph, showing terminal conduit portion of nephridium in cross section of body.  $\times 200$ . *a* ampulla, *cd.p.n* terminal conduit portion of nephridium, *lm* longitudinal muscle layer of ventral body wall, *v.b.w* ventral body wall.

The posterior portion of the nephrostome narrows, and passes through the posterior septum, and continues on to the slender neck portion of the nephridium, which involves only a single nephridial duct, and occupies the anterior space of the segment. This portion, extending upwards, carries the duct to that of the coiled portion (Fig. 24), which on its way increases

slightly in calibre and is situated on the dorso-lateral side of the intestine, inside the peritoneal cell-mass of the nephridium. In the coiled portion, at first, the nephridial duct proceeds posteriorly making manifold windings. Then, it comes back along the former course, so that the nephridial duct is always observed to be double in this portion. At the end of this

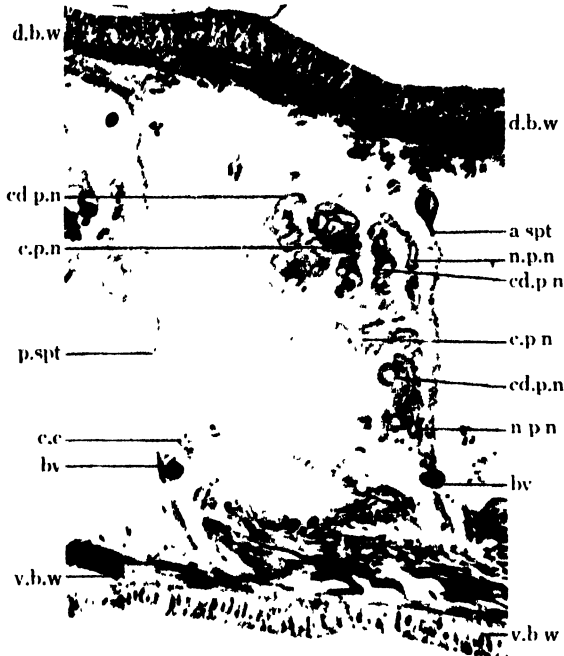


Fig. 24. Photomicrograph, showing nephridial duct in sagittal section of mature specimen  $\times 50$ . *a.spt* anterior septum, *b.v* blood vessel, *c.c* coelomic corpuscle, *cd.p.n* conduit portion of nephridium, *c.p.n* coiled portion of nephridium, *d.b.w* dorsal body wall, *n.p.n* neck portion of nephridium, *p.spt* posterior septum, *v.b.w* ventral body wall.

being flattened laterally. Finally, the thickened conduit portion opens into the ampulla, which is a dilated terminal of the nephridial duct. This ampulla, in turn, opens into the exterior just in front of the ventral seta-bundle.

In the immature specimens, pairs of the peritoneal cell-masses, similar to those of the nephridia, are observed in Segments VIII–XIV (Fig. 25), and, even in the mature specimens, such peritoneal cell-masses, but of small size, are often found arranged on a level just outside the gonadal

course, a single duct comes out apart from the coiled portion and approaches the neck portion. The single duct turns then postero-ventrally and continues to that of the conduit portion (Fig. 23), which increases in calibre several times in comparison with that of the neck portion, and traverses the peritoneal cell-mass of the nephridium making a few convolutions along its antero-ventral and ventral margins. The cell-mass lies on the lateral side of the intestine, partly covering the coiled portion, which is directly dorso-lateral to the intestine, and attains a considerable size,

lines on both sides of the body.

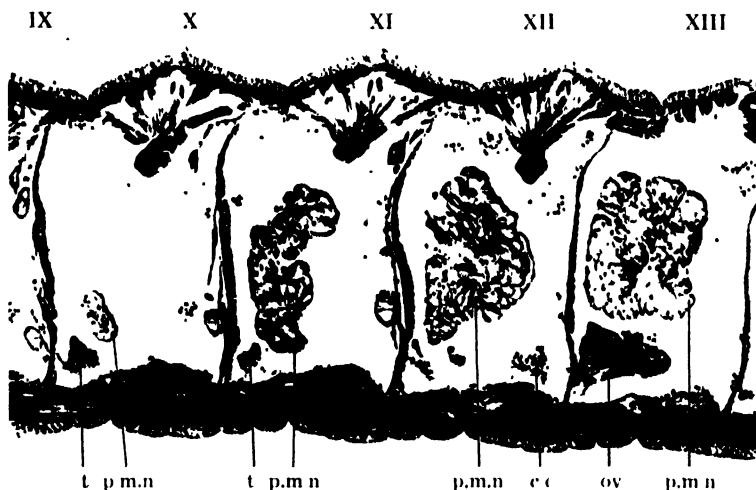


Fig. 25 Photomicrograph, showing arrangement of gonads and of peritoneal cell-masses similar to those of nephridia in sagittal section of immature specimen  $\times 50$  c.c coelomic corpuscle, p.m.n peritoneal cell-mass similar to that of nephridium, ov ovary, t testis, IX XIII Segments IX XIII.

#### THE VASCULAR SYSTEM

The vascular system of the species under investigation consists mainly of a dorsal, a ventral, a supra-intestinal, and a sub-intestinal vessel on the median plane, and of two pairs of lateral vessels in the anterior segments on both sides of the body. Besides these, there are a number of vertical connectives between the dorsal and the supra-intestinal vessels, and between the ventral and the sub-intestinal vessels, and, in each segment, a pair of lateral commissures and of lattice works of the intestinal vessels.

1) The dorsal vessel. This vessel runs from the anterior to the posterior end of the body on the dorsal side of the alimentary canal.

Just in front of the brain, the anterior end of the dorsal vessel is divided into two branches, which enter the prostomium. After the branches divide, the dorsal vessel proceeds posteriorly along the ventral surface of the brain. Then, running postero-dorsally, it passes by the perforation of the brain, and continues on a backward course apart from the pharyngeal endoderm, which is here joined by the ducts of the pharyngeal glands and other tissues, but, posterior to Segment V, it comes near the endoderm,

and, in the posterior portion of the body, the dorsal vessel is attached directly to the dorsal wall of the intestine.

The dorsal vessel posterior to Segment IV is surrounded by chloragogen cells. Thus, the walls of the dorsal vessel consist, for the most part, of a layer of chloragogen cells, a layer of contractile fibres, a circular muscle layer, a longitudinal muscle layer, and an endothelium in the order of from outside to inside.

Moreover, in the dorsal vessel, syncytial, membranous valves are found forming sets. Each set consists of a pair of semicircular valves, and is

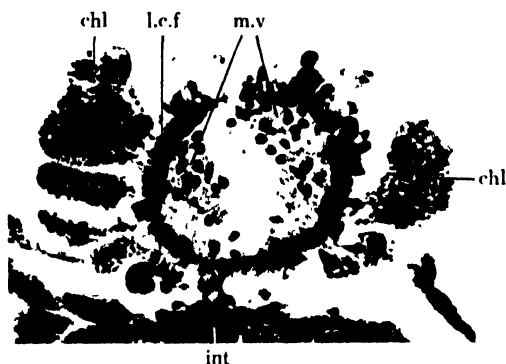


Fig. 26 Photomicrograph, showing structure of membranous valve and of walls of dorsal vessel in cross section of body through Segment XIV, just in front of posterior septum  $\times 200$  *chl* chloragogen cell, *l.c.f* layer of contractile fibres, *int* dorsal wall of intestine, *m.v* membranous valves

situated in every segment at a level just behind the branching of the lateral commissures near the anterior face of the posterior septum. Each membranous valve contains thin cytoplasm and many ellipsoidal nuclei (Fig. 26).

## 2) The ventral vessel.

This vessel also runs through the whole length of the body on the ventral side of the alimentary canal and, consequently, on the

dorsal side of the ventral nerve cord, but sometimes having a slightly irregular course.

The ventral vessel is narrow in comparison with the dorsal vessel. It begins, anteriorly, at the posterior end of Segment II, i.e. at the point of union of the pair of branches from the prostomium.

The walls are very thin, the layer of contractile fibres and of chloragogen cells being completely lacking, and the layers of muscle fibres being very slightly developed.

The ventral vessel is entirely destitute of valvular cells.

3) The supra-intestinal vessel. This vessel lies attached directly to the dorsal surface of the alimentary canal, and is distinct in the segments anterior to IX.

4) The sub-intestinal vessel. It is difficult to call this a distinct vessel,

but it may be traced, being attached to the ventral face of the alimentary canal in the same segments as that in which the supra-intestinal vessel occurs.

5) The vertical connectives. In each segment, in which either the supra-intestinal or the sub-intestinal vessel is present, from one to five vertical connectives are found between the dorsal and the supra-intestinal vessels, and one connective between the ventral and the sub-intestinal vessels near the anterior face of the posterior septum.

6) The lateral vessels. These are a dorsal and a ventral pair of vessels which run longitudinally in the coelom of the segments anterior to XIV, on both sides of the body, along the lateral lines. The dorsal pair is somewhat thicker than the ventral.

The dorsal lateral vessel starts from the dorsal vessel, and the ventral lateral one from the sub-intestinal vessel, respectively, at a level near the anterior face of Septum XIV/XV. They both proceed laterally at first and then anteriorly, and reach as far as the prostomium, giving off branches, in each segment, to the dorsal and the ventral body walls.

7) The lateral commissures. The lateral commissures in the segments anterior to XIV are perfectly coelomic and are destitute of branches (Fig. 28), starting from the dorsal vessel, and ending in the ventral vessel, at the posterior region of a corresponding segment. In these segments, the branchlets from the lateral vessels form the ramifications of the blood capillaries of the body wall.

From the lateral commissures in the segments posterior to XIV issue many branches, the branchlets of which form ramifications of the blood capillaries of the body wall. Both the efferent and afferent trunks of each commissure start from the dorsal and ventral vessels, respectively, in the posterior region of a corresponding segment (Fig. 29).

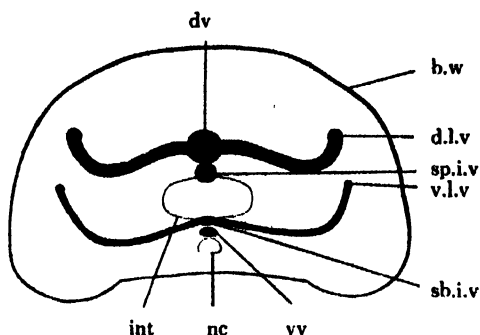


Fig. 27. Diagrammatic representation of lateral vessels, showing relation to dorsal vessel and to sub-intestinal vessel in imaginary cross section of body through portion in front of posterior septum of Segment XIV. *b.w* body wall, *d.l.v* dorsal lateral vessel, *dv* dorsal vessel, *int* intestine, *nc* ventral nerve cord, *sb.i.v* sub-intestinal vessel, *sp.i.v* supra-intestinal vessel, *v.l.v* ventral lateral vessel, *vv* ventral vessel.

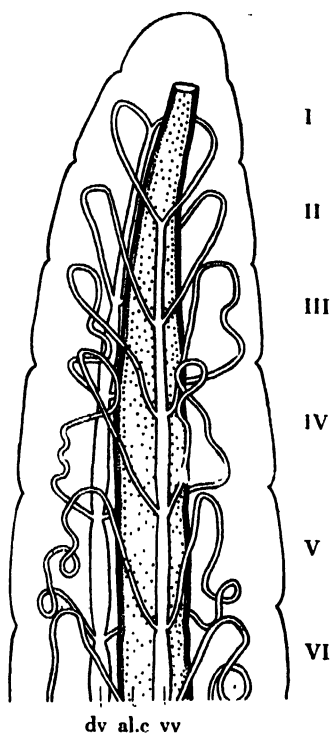


Fig. 28. Diagrammatic representation of dorsal and ventral vessels and lateral commissures, showing relation between them in immature specimen, *a.l.c.* alimentary canal, *dv* dorsal vessel, *vv* ventral vessel.

the "hearts," because of their specialized structure. The walls of the branchless commissure are constructed similarly to those of the dorsal vessel, and each of the hearts is furnished with three or more sets of valves. From the fact that the valves are also present in the lateral commissures in Segments V and VI, it may be considered that the species under discussion may have seven pairs of hearts.

Each heart is comparatively narrow when it branches from the dorsal vessel, but, proceeding postero-dorsally, it soon increases in thickness, and reaches the middle portion of the posterior half of a segment at a level just inside the corresponding dorsal setal line, where the heart is hung from the dorsal wall of the body by muscle fibres originating from the corresponding septum and body wall. Then, the heart undergoes a

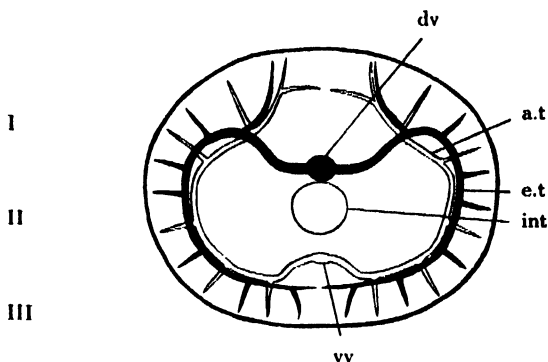


Fig. 29. Diagrammatic representation of lateral commissures in segment posterior to XIV, showing their course in cross section of body. *a.t.* afferent trunk of lateral commissure opening to ventral vessel, *dv* dorsal vessel, *e.t.* efferent trunk of lateral commissure originating from dorsal vessel, *int* intestine, *vv* ventral vessel.

8) The intestinal lattice works. These works are well developed round the intestine. In the anterior segments, they are found between the supra-intestinal and sub-intestinal vessels, but in the posterior segments, between the dorsal and ventral vessels.

9) The hearts. In the present paper, the five pairs of lateral commissures in Segments VII–XI are called

slight convolution and proceeds postero-ventrally and finally opens into the ventral vessel just in front of the anterior face of the posterior septum. In the portion near the ventral end, the walls of the heart lack the layers of chloragogen cells and contractile fibres as well as the muscle layers.

In Segments VII and VIII, each heart is connected dorsally by the muscle fibres, which also connect with the corresponding pharyngeal gland. Each heart in Segment IX convolutes round the outer surface of the corresponding anterior sperm sac, and that in Segments X and XI passes just behind the sperm-duct funnel before its union with the ventral vessel.

#### THE GENITAL SYSTEM

There are two pairs of testes and of sperm-duct funnels in Segments X and XI; two pairs of sperm sacs in Segments IX and XII; two pairs of sperm ducts in Segments XI-XIII; one pair of ovaries, of oviduct funnels, of male pores, of copulation glands, and of prostate glands in Segment XIII; and one pair of ovisacs, of oviducts and of female pores in Segment XIV (Fig. 30). All the genital organs of the species in question are ventro-lateral to the alimentary canal inside the ventral setal lines, with the exception of the anterior pair of sperm sacs, which are dorso-lateral to it, and of the male pores, the copulation glands and the prostate glands, which are located in the lateral swellings.

1) The testes. The anterior pair of testes are attached to the posterior face of Septum IX/X, and the posterior pair to that of Septum X/XI. When the worms attain sexual maturity, the segments of the tests, viz. Segments X and XI, are filled with male cells.

2) The sperm sacs. The anterior pair of sperm sacs are the anteriorly directed bulges from Septum IX/X into the coelom of Segment IX, and are situated dorso-laterally to the dorsal vessel. The entrance to each sac is comparatively narrow, and adjoins the dorsal body wall, the spacious, spherical, saccular portion being hung antero-ventrally (Fig. 31).

The posterior pair of sperm sacs are the posteriorly directed bulges of Septum XI/XII into the coelom of Segment XII (Fig. 32). The narrow entrance to each posterior sperm sac is situated slightly inside, and dorsal to, the sperm duct on either side of the body.

Both vesicles of either anterior or posterior pair stand usually apart, but when they attain full development, they are found frequently in close contact with each other. Sometimes, their distal end is divided into two



or more saccules, probably owing to the presence of muscle fibres extending from the dorsal body wall. The walls of each vesicle are very thin, consisting of a single unicellular layer of flattened cells, but those at the entrance are thick, showing a similar structure to the neighbouring septum.

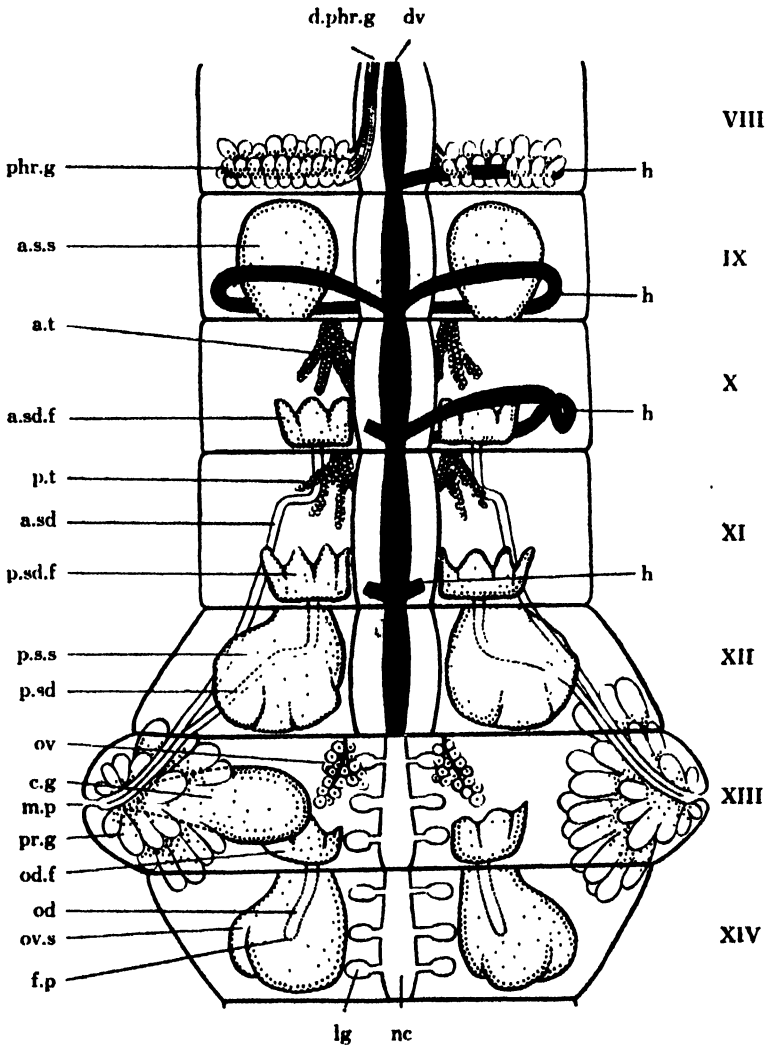


Fig. 30. Diagrammatic representation of genital organs, showing their arrangement. *a.s.d* anterior sperm duct, *a.s.d.f* anterior sperm-duct funnel, *a.s.s* anterior sperm sac, *a.t* anterior testis, *c.g* copulation gland, *dv* dorsal vessel, *f.p* female pore, *h* heart, *m.p* male pore, *od* oviduct, *od.f* oviduct funnel, *ov* ovary, *ov.s* ovisac, *phr.g* pharyngeal gland, *pr.g* prostate gland, *p.s.d* posterior sperm duct, *p.s.d.f* posterior sperm-duct funnel, *p.s.s* posterior sperm sac, *p.t* posterior testis.

3) The sperm ducts. Each sperm duct consists of a widely opened funnel and a long, slender duct portion.

a. The sperm-duct funnels. While the worms are immature, each sperm-duct funnel is nearly a conical mass of cells, attached by its narrow end to the anterior face of the posterior septum of Segment X and of Segment XI. In the course of development, the central axis of the cone acquires a lumen, and the walls round the lumen become irregularly folded (Fig. 31). When fully developed, the entire funnel has an urceolate shape with its much folded, wider dorsal and less folded, narrower ventral walls, its centre being located eccentrically. It sometimes reaches the middle of the corresponding segment, and occupies a quarter of the coelom in cross sections of the segment, on the lateral side of the alimentary tract and ventral nerve cord. The portion of each funnel, just ventral to the opening of the duct portion, is invariably folded transversely, owing to the transverse course of the corresponding heart along the posterior surface of the funnel.

The walls of the sperm-duct funnel consist of two

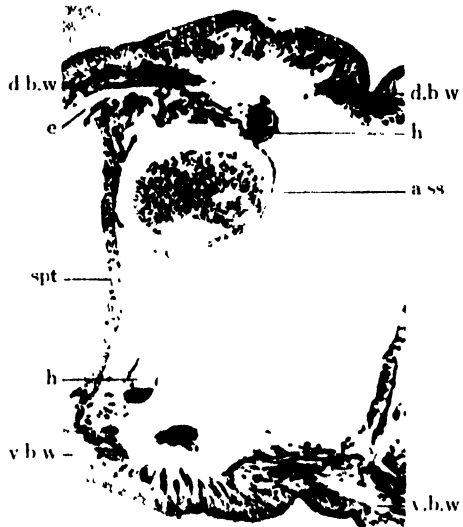


Fig. 31. Photomicrograph, showing anterior sperm sac and adjacent organs in sagittal section of body  $\times 50$ . *a.s.s.* anterior sperm sac, *d.b.w.* dorsal body wall, *e* entrance to anterior sperm sac, *h* heart, *spt* Septum IX/X, *v.b.w.* ventral body wall.

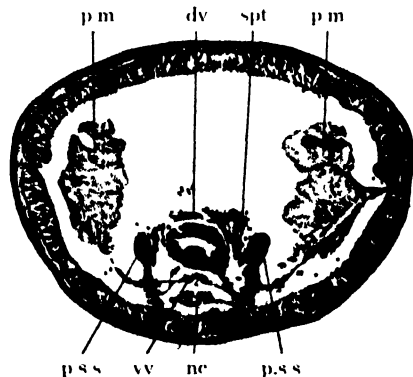


Fig. 32. Photomicrograph, showing posterior sperm sac at early stage of development and adjacent organs in cross section of immature specimen through Septum XI/XII.  $\times 50$ . *dv* dorsal vessel, *nc* ventral nerve cord, *p.m.* peritoneal cell-mass similar to that of nephridium, *p.s.s.* posterior sperm sac, *spt* Septum XI/XII, *vv* ventral vessel.

layers: an inner layer of ciliated columnar cells and an outer layer of flattened peritoneal cells mingling with slightly developed muscle fibres. Each columnar cell of the inner layer is almost  $12\ \mu$  by  $10\ \mu$ , and contains a quantity of vacuolated cytoplasm, and a spherical or an ellipsoidal nucleus at its proximal half. The cilia measure about  $14\text{--}15\ \mu$  in length.

*b.* The duct portions. Immediately after the beginning at the posterior end of the respective funnel, each duct portion of the sperm duct passes through the corresponding septum and proceeds into the coelom of the next segment.



Fig. 33. Photomicrograph, showing anterior sperm-duct funnel, posterior testis, and adjacent organs in sagittal section of body.  $\times 100$ . *a.s.d.f* anterior sperm-duct funnel, *h* heart, *m.c* male cells, *p.t* posterior testis, *spt* Septum X/XI, *v.b.w* ventral body wall.

Each duct portion of the anterior pair passes through Septum X/XI and runs backwards parallel to the body wall for a little distance along a longitudinal line outside the point of attachment of the posterior testis on either side of the body. Proceeding then obliquely outwards towards the outside of the ventral seta-bundle, it makes a few slight windings freely in the coelom of Segment XI, and enters the longitudinal muscle layer of the body wall at a level anterior to the corresponding ventral setae. Then, it proceeds postero-exteriorly and, finally, entering the hypodermal layer of the body wall at a level a little in front of Septum XI/XII, it still continues on an obliquely backward course in the hypodermis of Segment XII.

Each duct portion of the posterior pair passes through Septum XI/XII on the ventro-exterior side of the corresponding sperm sac. After proceeding also on its backward course in the coelom of Segment XII, as in the case of that of the anterior pair, it enters the hypodermis at the anterior base of the lateral swelling or at the middle of Segment

XII, on either side of the body.

Both the anterior and posterior duct portions come into contact parallel to each other, the anterior one being arranged dorsally and the posterior one ventrally, in the hypodermis on the dorso-anterior slope of the lateral swelling, at a level between the lateral line and the ventral setal line, on either side of the body. Then, the adhering duct portions proceed ventro-laterally and posteriorly, and open at last, obliquely from the antero-dorsal direction, to the common male pore, which is situated in the vertical slit between the papillae of the lateral swelling.

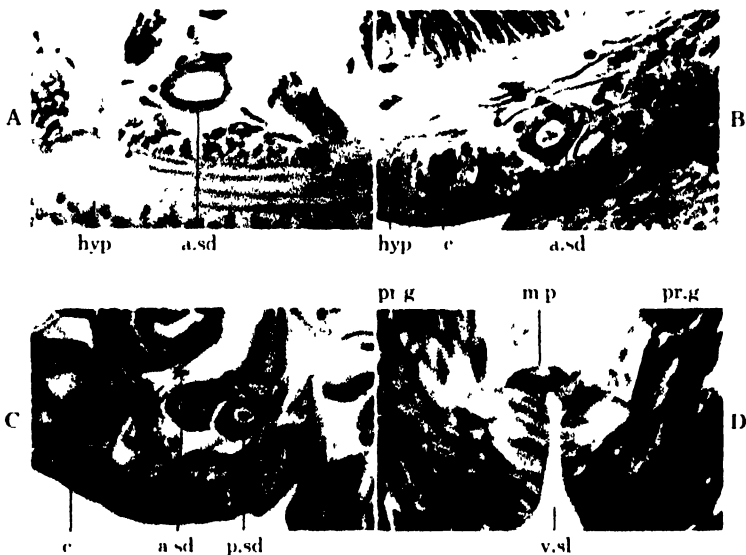


Fig. 34. Photomicrograph, showing relation of duct portion of sperm duct to other tissues.  $\times 200$ .

A Cross section of anterior sperm duct in coelom

B Cross section of anterior sperm duct in hypodermis.

C Cross section of both anterior and posterior sperm ducts arranged in close contact in hypodermis.

D Cross section of common male pore.

a.s.d anterior sperm duct, c cuticle, hyp hypodermis, m.p common male pore, pr.g prostate gland, p.s.d posterior sperm duct, v.sl vertical slit.

Here, it will be interesting to compare the sperm ducts of the species under investigation with those of *Criodrilus lacuum* HOFFM. In the case of the latter species, ÖRLEY (1887) states that "The sperm ducts are spirally coiled at the base of the rosettes, unite with one another at the level of the somites XII and XIII, and thence a wider tortuous, common

canal extends on each side to the external pore on the ventral surface of the somite XV, between the two couples of setae."

Each duct portion narrows posteriorly. Actually, at the level where it just passes through the septum, it measures  $45\mu$  in diameter and  $25\mu$  in calibre. At the level of the entrance to the hypodermis, it is  $37\mu$  in diameter and  $17\mu$  in calibre, and in the greater part of the hypodermis, the diameter is less than  $23\mu$  and the calibre  $13\mu$  or so. At a level near the common male pore, it finally measures only  $17\mu$  in diameter and  $8\mu$  in calibre.

Two layers of the sperm-duct funnel continue directly to those of the duct portion, the muscle fibres of the outer layer being almost obscured, and the inner layer showing a syncytial appearance. At the beginning of each duct portion, the inner layer, in circular cross section, contains five



Fig. 35. Photomicrograph, showing relation of common male pore to neighbouring organs in cross section of body through vertical slit at apex of lateral swelling.  $\times 100$ . *a.s.d* anterior sperm duct, *b.c* blood capillaries, *c.g* copulation gland, *d.c.g* duct of copulation gland, *g.s.b* genital seta-bundle, *m.p* common male pore, *p.s.d* posterior sperm duct, *s.v.sl* space of vertical slit between papillae.

or more nuclei, but the number of nuclei decreases with the narrowing of the diameter of the duct, and in the terminal portion near the common male pore it finally contains only one or two.

4) The common male pores. Each common male pore is a small

conical cavity, which receives, at its narrower end, two lumens of the adhering sperm ducts, and opens, at its wider end, to the vertical slit between the anterior and posterior papillae at the apex of either lateral swelling. Its opening is in the dorsal direction in the dorsal portion of the slit (Figs. 34 D and 35).

5) The prostate glands. On either side of an immature specimen, which shows no signs of lateral swellings, many slender fusiform cells are found attached to a portion of the area of the inner surface of the ventral

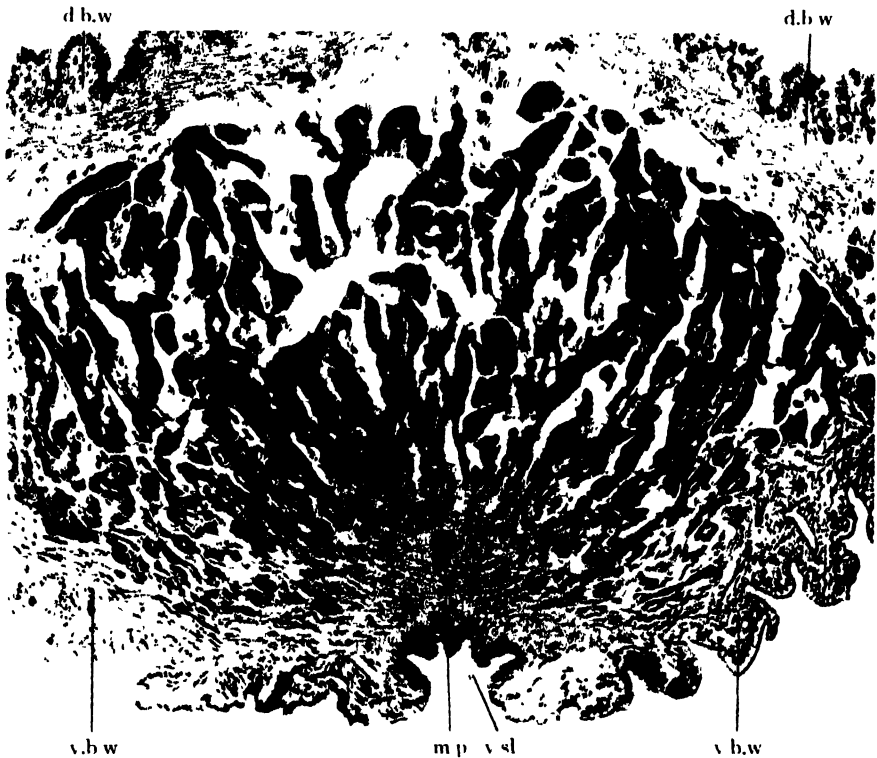


Fig. 36 Photomicrograph, showing arrangement of prostate gland cells in sagittal section of body.  $\times 50$ . *d.b.w* dorsal body wall, *m.p* common male pore, *v.b.w* ventral body wall, *v.sl* vertical slit.

body wall in Segment XIII, which corresponds to the basal area of the future lateral swelling. Each of these cells is nearly  $21\mu$  in length and  $4\mu$  in width, containing a fusiform, compact nucleus and uniformly distributed granules. With the progress of the development of the musculature of the lateral swelling, these cells become attached to the

muscle fibres, and, later, even to the blood capillaries in connection with the musculature. Then, with the bulging out of the lateral swelling, these cells begin to increase in bulk and to assume a pear shape. The nuclei alter in outline from fusiform to spherical, and come to contain, respectively, a large nucleolus and diffused chromatin granules. Not only these latter, but the exterior ends of these cells begin to elongate and form excretory ducts. When these cells attain full development, each measures nearly  $32\ \mu$  in width and  $75\ \mu$  in length, with the exception of the portion of excretory duct, the length of which is indeterminable, and the diameter of which is  $5\ \mu$ . The nuclei are generally roundish, measuring  $10\ \mu$  in diameter and the nucleoli  $2.5\ \mu$ .



Fig. 37. Photomicrograph, showing copulation gland and adjacent organs in sagittal section of body.  $\times 50$ . *c.g* copulation gland, *g.s* genital setae, *ov* ovary, *ovd* oviduct, *pr.g* prostate gland.

Such cells are aggregated into lobules of the prostate gland. Some of the excretory ducts of these gland cells are usually united to form a common duct. Bundles of ducts as well as common ducts run centripetally, respectively, through the central axis of the lobule, towards the

common male pore, and open into the hypodermis of the vertical slit, just ventral to the common male pore.

The excretory granules contained in these gland cells are seemingly of equal size, but, usually, they are aggregated and form globules of different size in several places in the cell. The largest globule found in an excretory duct is nearly the same in size as that found in a gland cell.

6) The copulation glands. The technical term, copulation gland, may not be the correct one to use for this organ, but we venture to use it, following "Copulationstasche" by MICHAELSEN (1918), in our sense, viz. that the secretion from the gland cells of this organ may probably be used in copulation and form spermatophores, being mixed with that from the prostate gland cells.

Each of these organs is a large cylindrical gland on either side of the body, and opens exteriorly to the vertical slit between the papillae of the lateral swelling in Segment XIII, ventrally to the portion, where the ducts of the prostate gland open, and just exteriorly to the genital setae.



Fig 38. Photomicrograph, showing double occurrence of copulation glands in sagittal section of body.  $\times 50$ . *c.g.XIII* copulation gland in Segment XIII, *c.g.XIV* copulation gland in Segment XIV, *d.b.w* dorsal body wall, *pr.g* prostate gland, *v.b.w* ventral body wall.

It is worthy of note that, in one immature specimen, I was able to find a case of double occurrence of the copulation glands (Fig. 38). One pair was in Segment XIII as usual, and there was another pair in Segment XIV. In the latter segment, the prostate glands were developed normally, but there was no trace of sperm ducts nor of lateral swellings. Even in this double case, the sperm duct opened in connection with the lateral swelling in Segment XIII.



In immature specimens, each organ comes into view as a small, saccular swelling from the hypodermis, in the ventro-lateral body wall, standing amidst the assemblage of the fusiform cells, i. e. the rudimentary prostate gland cells (Fig. 39).

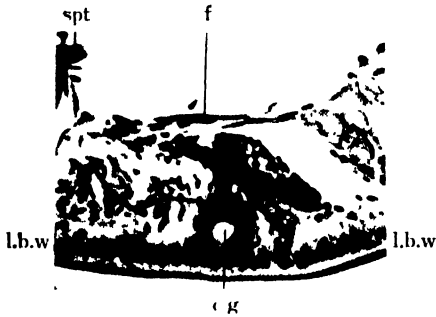


Fig. 39 Photomicrograph, showing rudimentary copulation gland and adjacent tissues in cross section of immature specimen  $\times 200$ . *c.g.* rudimentary copulation gland, *f* fusiform cell or rudimentary prostate gland cell, *l.b.w.* ventro-lateral body wall, *spt* Septum XIII/XIV.

With the bulging out of the lateral swelling, on either side of the body, the saccular swelling increases in bulk, and becomes elongated along with its inner cavity.

When its diameter reaches about  $45\mu$ , the copulation gland is completely developed. This fully grown gland runs dorso-medially, beginning at the external margin of the lateral swelling, nearly along its central axis, winding slightly twice or more. When the copulation glands attain full growth, their diameter increases and is as wide as  $260\mu$  or more, and a meeting of the distal blind ends of these organs may often be observed on the median plane of the body in the coelom, dorsally to the dorsal vessel.

The inner cavity, which is the excretory duct of the full-grown copulation gland, measures  $14\mu$  in caliber near its external opening, and, the calibre increasing distally, the cavity finally measures from  $35\mu$  to  $60\mu$  in the middle portion, and in its largest calibre then continues to the distal blind end.

Each copulation gland may be divided into two portions: a proximal duct portion and a distal glandular portion.

The innermost columnar layer of the walls of the duct portion is distinctly a continuation of the hypodermis of the body wall. Each columnar cell, forming this layer, is about  $15\mu$  by  $10\mu$  and contains compact cytoplasm, which is as intensely stainable as that of the hypodermis.

The walls of the glandular portion consist of three layers: an outer peritoneal layer, a middle muscle layer containing blood capillaries, and an inner glandular layer (Fig. 10). Both muscular and peritoneal layers are very slightly developed anywhere in this portion.

The inner layer of the glandular portion consists of three kinds of

cells, viz. a club-shaped cell with a nucleus near its distal end, a fusiform cell with a nucleus near its centre, and a cuneiform cell with a nucleus

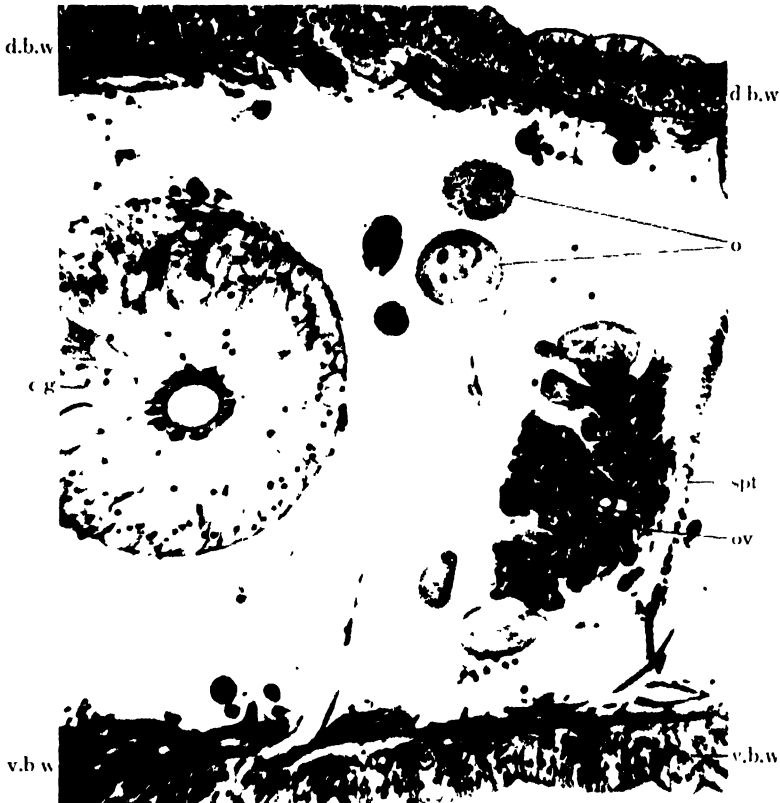


Fig. 10. Photomicrograph, showing copulation gland and ovary in sagittal section of body.  $\times 100$ . *cg* copulation gland in cross section, *dbw* dorsal body wall, *o* ovum, *ov* ovary, *vbw* ventral body wall, *spt* Septum XII/XIII

at its centre. Among these cells, the club-shaped cells are the largest and the cuneiform cells the shortest, the fusiform ones being of an intermediate length between the above two. The distal ends of the club-shaped cells are arranged at the outermost, the cuneiform cells at the innermost, and the fusiform cells at the middle stratum, so that the glandular layer is apparently a multicellular layer, notwithstanding its original unicellular structure.

In reality, in the full-grown specimens, the inner epithelium of the duct portion continues for nearly  $70\ \mu$  in distance and is suddenly transferred to the thickened layer of the gland cells of the glandular portion

(Fig. 35 c. g and d. c. g). In one case, the hypodermal structure of the duct portion continued for more than  $150\mu$ , and, at the level of from  $70\mu$  to  $150\mu$ , the walls were constructed of four layers: a hypodermal, a glandular, a muscular and a peritoneal layer, in the order of from inner to outer. This structure definitely reveals the existence of a transitional stage from hypodermal cells to gland cells, and, therefore, the purely ectodermal origin of the inner lining of the copulation gland.

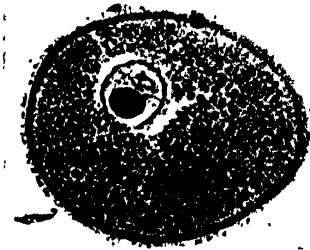


Fig 41 Photomicrograph, showing ovum freed in coelom and surrounded by spermatozoa.  $\times 600$ .

7) The ovaries. They are attached to the posterior face of Septum XII/XIII on the same longitudinal lines as the testes. In the early stage, the ovary is nearly conical, but later it becomes divided into lobes. Free ova are frequently observed in the coelom of Segment XIII in sexually mature worms.

8) The ovisacs. The paired ovisacs are the posteriorly directed bulges from the posterior septum of Segment XIII. The entrance to each ovisac is situated dorsally to, and slightly inside, the oviduct.

The walls of the ovisac consist of a unicellular layer of flattened cells, but those of the entrance, which adjoin the septal wall, are somewhat thickened owing to the addition of the peritoneal cells. In sexually mature specimens, the ovisac often approaches near the posterior septum of Segment XIV.

9 The oviducts. Each oviduct consists of a widely opened funnel and a duct portion.

The oviduct funnel is similarly constructed as in the case of the sperm-duct funnel. In this case, however, the funnel is attached to the anterior face of Septum XIII/XIV, and the foldings of its walls are less in number than those of the sperm-duct funnel.

The duct portion runs backwards beginning at the posterior end of the funnel. It is also constructed similarly to that of the sperm duct, but, in this case, the outer peritoneal layer contains distinctly developed muscular fibres. After running posteriorly and ventro-exteriorly, the duct portion opens to the exterior just in front of the ventral setae, through the clitellar hypodermis, which begins slightly anterior to the female pore.

10) The spermatophores. The spermatophores are brownish yellow in colour. They are attached, mostly in pairs, to the outer surface of

the body (Fig. 12) in the neighbourhood of the male pores, especially on the dorsal and dorso-lateral body walls (Fig. 43), but rarely on the ventral body wall posterior to the male pores. ÖRLEY (1887) states that *Criodrilus lacuum* has extraordinarily tubular spermatophores, usually attached to the ventral body wall, and that the spermatophores are found always in pairs in the region of Segment XIII anterior to the male apertures. According to BEDDARD (1901) and to STEPHENSON (1910), in *Bothrioneurum iris*, the spermatophores are attached not only to the ventral body wall, but also to the dorsal and dorso-lateral body walls.

Each spermatophore measures 0.6–0.7 mm. in total length, consisting of a stalk and a saccular portion. The stalk portion is attached to the cuticular layer of the body wall with its discoidally expanded, proximal base, which is nearly 0.5 mm. in diameter when in a fresh condition. This discoidal portion narrows distally, and, at its distal end, it continues to a hollow, fusiform, saccular portion. This saccular portion is nearly 0.45 mm. in height and 0.25–0.30 mm. in width, with its apex somewhat pointed. The sperm chamber, which is enclosed in the saccular portion is nearly ovoid. The spermatozoa show a parallel, but somewhat spiral, arrangement round the long axis of the hollow space.

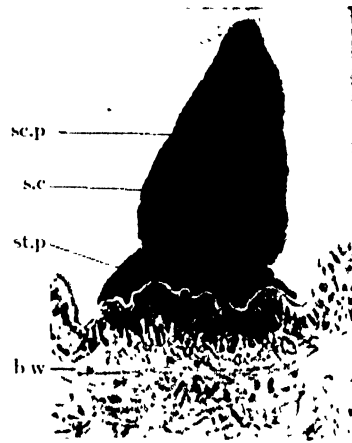


Fig. 12. Photomicrograph, showing spermatophore attaching to body wall in cross section of body  $\times 100$ . *b.w* body wall, *s.c* sperm chamber, *sc.p* saccular portion of spermatophore, *st.p* stalk portion of spermatophore

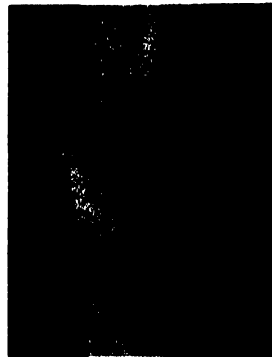


Fig. 13. Photomicrograph, showing spermatophores in pairs, attached to dorsal body wall in neighbourhood of lateral swellings.  $\times 10$ .

#### REMARKS AND CONCLUSION

In our sexually mature specimens also, the dorsal pores, the gizzard

and the calcareous glands are absent, and the lateral lips in the anus and the lateral vessels in the anterior part of the body are present as in the case of immature specimens of *Criodrilus bathybathe* STEPHENSON.

"In each nephridium," states STEPHENSON, "two parts can be seen, one a somewhat flattened, lobed, opaque white mass near the external end of the organ, the other a twisted tube extending inwards towards the middle line." If we assume that the white mass and the twisted tube are, respectively, identical with the peritoneal cell-mass and the combined part of the neck and coiled portions mentioned in our description, not only this, but also other statements of his in relation to the nephridia are directly applicable to our specimens.

As to the apparent differences between his and our specimens, we are of opinion that these may depend upon differences in the developmental stages. For example, the clitellum, which is described as non-existent, and the pair of sperm sacs in Segment IX, which are not observed by STEPHENSON, might have subsequently developed, if his specimen had been fully matured.

"What is perhaps a pair of 'prostate' glands," states STEPHENSON, "is present in Segment XIII; each is a small and narrow white transversely elongated structure, resting on the body wall throughout its length and attached at its inner end; they were situated nearer the posterior than the anterior limit of the segment, and occupies the middle of the interval between the lines of the dorsal and ventral setae, — their inner ends nearer to the line of the ventral setae than the outer ends to the dorsal setae; the inner end of each appeared to correspond in position to the centre of the male papilla." His 'prostate' glands probably correspond to our copulation glands.

The greater part of the structure of our specimen may, thus, be considered to be identical with that of STEPHENSON's *Criodrilus bathybathe*, but the difference between the depths of the habitats and between his setae and ours must be considered most important, and it is these difficulties that make what let us feel the difficulty of identifying both specimens with each other.

Our specimens were collected at a depth shallower than 5 feet or so, but STEPHENSON's specimens were from a depth of 180 feet. In both cases, however, the habitat was mud mixed with stones.

According to STEPHENSON, in the case of his species, the setae are small and closely paired. The 'smallness' and 'closeness' are not distinctly evident to us. In our specimens, the dorsal are larger than the

ventral setae, and the ventral are longer than one-fifth of the depth of the body in the middle region of the body. The angular divergence between the two setae in a bundle is  $30^{\circ}$ – $45^{\circ}$ : it is wider in the anterior segments than in the posterior.

Moreover, STEPHENSON states that there are no ventral setae in Segment XIII, and that there are no other genital marks. In our specimen, the ordinary ventral setae are non-existent in Segment XIII, but the genital setae are, as far as our observation goes, found there.

Furthermore, KAWAMURA (1918) states that *Criodrilus bathybathe* lives in mud at the bottom of Lake Biwa and produces big cocoons, which taper towards both ends, and that each cocoon contains a number of the eggs. We earnestly desired, of course, to obtain the cocoons in both localities, Kômorimachi and Tsuruoka, and to compare them with those of Kawamura. Samples, however, were not obtainable, and Mr. TSUJI and Mr. NODA, to whom we applied for collection, report to us that no cocoons were to be found after all.

We cannot expect to have any opportunity of collecting *Criodrilus bathybathe* STEPHENSON from the bottom of Lake Biwa and so as to compare both species, STEPHENSON's and ours. Therefore, in the present paper, we are obliged to conclude that our species is closely allied to *Criodrilus bathybathe* STEPHENSON, but that the former species, which has genital setae, is different from the latter species, which has none. If our view be acceptable, we beg to propose as the new name *Criodrilus miyashitai*, in honour of Mr. YOSHINOBU MIYASHITA, who was the first discoverer of the present species.

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# INFLUENCE OF TEMPERATURE ON THE GROWTH OF *DROSOPHILA MELANOGASTER*

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(With two figures)

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## INTRODUCTION

The influence of temperature on the dimensional characteristics of *Drosophila melanogaster* has been a point of investigation by several biologists. ALFATOV and PEARL (1929), EIGENBRODT (1930) and IMAI (1933) have demonstrated that, with rising temperature, dimensions of such characters as wing and leg are diminished. According to the results of RIEDEL (1934) such rule holds at temperatures higher than 15°C. and below this temperature body size diminishes inversely. Such facts can be taken as an indication that the lower limit of the temperature range for the normal growth of *Drosophila* is around this point. IMAI (1934) demonstrated that the size of the egg is also influenced by the temperature to which the animal is subjected, in the same direction as is found in the adult forms.

These studies, however, concern only the final size of growth and no systematic study has been reported as to the thermal effect on dimensional characteristics during the periods of growth and development. Such study is undoubtedly important for the analysis of the nature of the thermal effect and also for the analysis of growth process in general.

There are two phases to be considered in regard to the growth and development of *Drosophila*, namely larva and pupa. For the larval stage it was demonstrated by ALFATOV (1929) that there are three cycles of growth, each of which shows a logistic curve.

The present study was planned to find the dimensional characteristics during the cycles of larval growth and also at the pupa stage under varying temperature conditions, and also to see how the thermal effect which would appear during growth periods will be related to those found

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in adult form. Though the size of the larvae varies from time to time, thermal effects can be seen by comparing the dimensions at the equilibrium phases of the growth cycles.

### MATERIALS AND METHODS

The material for this study was the normal wild type of fruit fly, *Drosophila melanogaster* which belonged to the same line I had used in previous experiments (1933 and 1934). Three groups of parents all arising from the same homogeneous strain were put under three different temperature conditions, 28°C., 25°C. and 18°C. Eggs were collected in watch glasses containing synthesized food (PEARL, ALLEN and PENNIMAN,

1926), for one, two and three hour periods respectively according to temperatures. Collections were repeated several times until there were enough eggs collected for the experiment. Watch glasses were put in sterilized Petri-dishes and were kept in the thermostats of the respective temperatures. Hatching occurred at roughly 18, 24 and 40 hours after the eggs were laid at the respective temperatures. Immediately after the larvae came out they were transferred into small bottles of 80 cc. capacity in which yeast had been grown on synthesized food two days previously. The number of larvae per bottle was 25. Every possible care was taken to minimize the

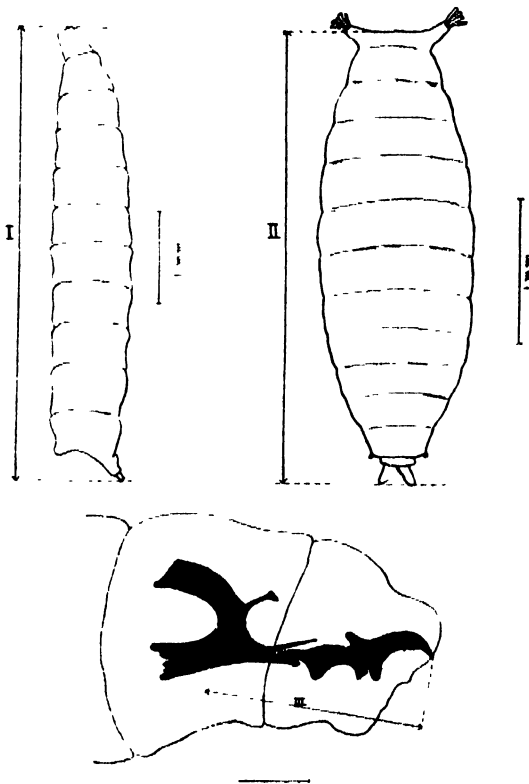


Fig. 1. Diagrams showing measured characters.

- I. Length of larva.
- II. Length of pupa.
- III. Length of mouth armature.

age variation of the larvae; it was within 20 minutes for 23°C. and even for the 18°C. series it was within 30 minutes. Every treatment was carried on under sterilized conditions.

Boiling hot water as devised by ALPATOV (1929) was used for killing the larvae, and they were preserved in 80% alcohol for measurement. Intervals between collections of the larvae were 5, 8 and 12 hours for the respective temperatures. Collections of pupae were carried on in the same way. Besides temperature, the relative humidity was also controlled and kept at 60%. Records showed that for temperature the maximum variation was  $\pm 0.4^\circ\text{C}.$ , and for humidity it was 6%.

The characters measured were, for larva, total length of body and length of mouth armature, and, for pupa, total length. These measured characters are shown in figure 1.

#### RESULTS OF EXPERIMENT

1. The results of measurements of larvae are shown in figure 2 and Table 1. As was already shown by ALPATOV (1929) there were three cycles of growth during larval life, and each cycle showed a logistic curve. Instars were easily distinguished by dimensions of mouth armature.

It is impossible with the present data to discuss the characteristics of growth curve in detail, as the points of observation are too few. However, what we are concerned with here is the comparison of the asymptotic values of the growth cycles and such purpose will be attained by fitting a simple logistic curve.

A simple logistic curve of the form  $y = C + \frac{K}{1 + e^{a+bx}}$  was fitted to each growth cycle and it seemed to be a good fit (Fig. 2). In this expression,  $y$  is the dimension in mm. and  $x$  the time in hours, and  $C$ ,  $K$ ,  $a$  and  $b$  are constants. In fitting the curve,  $C$  and  $K$  were first determined by trial substitutions of their values at each observed point in the formula  $\log \frac{(K+C)-y}{y-C}$  until a straight line relationship in graph was realized, and then  $a$  and  $b$  were determined by the least square method. Numerical expressions of the fitted curves are shown in Table 2.

For the lower asymptote of the first cycle of growth, ALPATOV (1929) took zero dimension, while in the present study it was put equal to .77 mm., .82 mm. and .85 mm. for the respective temperatures, because it seemed better to follow actual observations. Although the first growth cycle of the larvae is undoubtedly a continuation of the growth which takes place

inside of the egg, we are not certain as to its mode, and in view of the fact that at the time of hatching the larvae are exposed to different surrounding conditions it is doubtful whether larval growth continues its

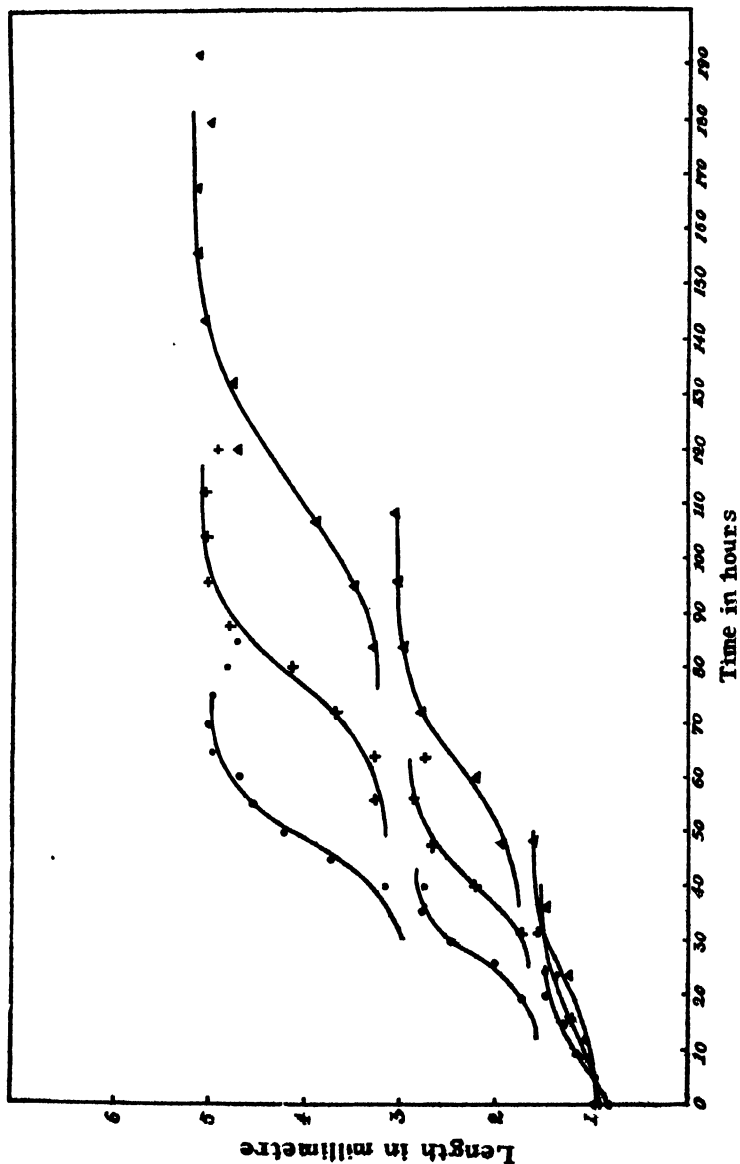


Fig. 2. Growth curves of the larvae.  
 • = 28°C. + = 23°C. ▲ = 18°C.

TABLE 1.

*Growth of larvae*

Stage	28°C.				23°C.				18°C.			
	Time in hrs.	Size of Sample	Body Length mm.	Length of Mouth Armature mm.	Time in hrs.	Size of Sample	Body Length mm.	Time in hrs.	Size of Sample	Time in hrs.	Size of Sample	Body Length mm.
I	0	40	.81±.005	.11±.0003	0	40	.86±.004	0	40	0	40	.89±.006
	5	40	.99±.008	.11±.0003	4	40	.99±.004	12	40	12	40	1.09±.007
	10	40	1.17±.008	.12±.0004	8	40	.94±.004	24	40	24	40	1.28±.007
	15	40	1.28±.007	.12±.0003	16	40	1.19±.004	36	40	36	40	1.51±.010
	20	37	1.43±.007	.11±.0003	24	40	1.33±.008	48	5	48	5	1.54±.022
	25	3	1.44		32	12	1.48±.013					
II	20	13	1.66±.014	.19±.0005	32	28	1.73±.014	48	40	48	40	1.86±.019
	25	40	2.04±.014	.19±.0007	40	40	2.22±.020	60	40	60	40	2.18±.019
	30	40	2.47±.018	.18±.0007	48	40	2.65±.018	72	40	72	40	2.72±.017
	35	40	2.73±.019	.18±.0008	56	36	2.84±.017	84	32	84	32	2.92±.017
	40	24	2.72±.042	.18±.0004	64	6	2.74±.014	96	32	96	32	3.01±.014
	45	2	2.65		72	3	2.70	108	7	108	7	3.07±.039
III	40	17	3.19±.056	.28±.0008	56	4	3.20±.041	84	8	84	8	3.28±.072
	45	40	3.67±.030	.29±.0009	64	35	3.27±.029	96	18	96	18	3.47±.064
	50	40	4.20±.045	.28±.0011	72	37	3.66±.038	108	40	108	40	3.91±.038
	55	40	4.52±.031	.28±.0010	80	40	4.07±.038	120	40	120	40	4.79±.034
	60	40	4.78±.040	.28±.0012	88	40	4.75±.027	132	40	132	40	4.80±.038
	65	40	4.90±.020	.29±.0006	96	40	5.05±.022	144	40	144	40	5.09±.027
	70	40	4.90±.041	.28±.0009	104	40	5.03±.032	156	40	156	40	5.12±.025
	75	40	4.91±.034	.29±.0012	112	30	5.03±.036	168	38	168	38	5.15±.029
	80	40	4.77±.040	.28±.0010	120	25	4.88±.040	180	37	180	37	5.02±.028
	85	24	4.69±.057	.28±.0017	128	6	4.65±.078	192	33	192	33	5.15±.028
	90	5	4.42±.132		136	6	4.90±.087	204	7	204	7	5.13±.079

TABLE 2.  
*Equations for larval growth*

Stage	Temperature	Equations of the logistic curve
I	28°C.	$y = .77 + \frac{.69}{1 + e^{2.390790 - .250293x}}$
	23°C.	$y = .82 + \frac{.70}{1 + e^{2.397697 - .147395x}}$
	18°C.	$y = .85 + \frac{.72}{1 + e^{2.526873 - .125434x}}$
II	28°C.	$y = 1.57 + \frac{1.25}{1 + e^{7.269896 - .261500x}}$
	23°C.	$y = 1.65 + \frac{1.26}{1 + e^{9.632697 - .226288x}}$
	18°C.	$y = 1.74 + \frac{1.29}{1 + e^{8.587547 - .217317x}}$
III	28°C.	$y = 3.00 + \frac{1.92}{1 + e^{10.512823 - .217317x}}$
	23°C.	$y = 3.11 + \frac{1.94}{1 + e^{13.383172 - .175458x}}$
	18°C.	$y = 3.22 + \frac{1.95}{1 + e^{10.897976 - .094752x}}$

dimensional increase at the pre-hatching rate.

2. As is seen from Table 2 and figure 2, increase of bodily dimension at the lower temperatures was already observed at hatching, and the same relation was found for asymptotic values of successive growths.

The dimension of mouth armature remained unchanged during each cycle as is shown in an example of the 28°C. series in Table 1. Biometric constants of the total measurements of mouth armature are summarized in Table 3. The comparison shows that they were influenced by temperature in the same direction as the body length of the larvae. The differences between varying temperatures were statistically significant.

The growth ratio of the mouth armature at moulting was found to be fairly constant in the different temperature series, and it was on the average 1.60 for the first moulting and 1.53 for the second. These values are close to the square of PRZIBRAM's factor,  $(\sqrt[3]{2})^2 = 1.59$ , and as ALPATOV stated, this fact may be taken to indicate that each of the moultings is connected with two cell divisions. However, it is to be noted that the ratio in the first moulting was significantly lower than that in the second, though their differences were small.

TABLE 3.  
*Biometric constants of the length of mouth armature*

Stage		28°C.	Difference	23°C.	Difference	18°C.
I	No.	179		169		164
	Mean in $\mu$ .	115.5 $\pm$ .15	2.6 $\pm$ .25	118.0 $\pm$ .20	3.5 $\pm$ .26	121.6 $\pm$ .17
	Standard deviation.	3.08		3.87		3.29
	Coefficient of variation.	2.67 $\pm$ .10		3.28 $\pm$ .12		3.71 $\pm$ .10
	Growth ratio.	1.61 $\pm$ .0036*		1.59 $\pm$ .0041		1.59 $\pm$ .0040
II	No.	156		150		188
	Mean in $\mu$ .	186.0 $\pm$ .34	2.0 $\pm$ .50	188.0 $\pm$ .37	4.8 $\pm$ .46	192.8 $\pm$ .27
	Standard deviation.	6.27		6.80		5.42
	Coefficient of variation.	3.37 $\pm$ .13		3.62 $\pm$ .14		2.81 $\pm$ .10
	Growth ratio.	1.53 $\pm$ .0034		1.54 $\pm$ .0031		1.53 $\pm$ .0043
III	No.	337		286		41
	Mean in $\mu$ .	283.7 $\pm$ .35	6.3 $\pm$ .46	290.0 $\pm$ .32	5.4 $\pm$ .82	295.4 $\pm$ .75
	Standard deviation.	9.77		8.39		7.12
	Coefficient of variation.	3.44 $\pm$ .09		2.89 $\pm$ .08		2.41 $\pm$ .18

\* *P.E.* is only given approximately, neglecting the coefficient of correlation which possibly exists between measurements of mouth armature at successive stages.

3. The length of the larvae at hatching were .81, .86 and .89 mm. for the respective temperatures and their differences were statistically significant (Table 1). Asymptotic values of fitted curves are summarized in Table 4.

TABLE 4.  
*Asymptotic values of growth curves*

Temperature	Stage I		Stage II		Stage III	
	Lower Asymptote in mm.	Upper Asymptote in mm.	Lower Asymptote in mm.	Upper Asymptote in mm.	Lower Asymptote in mm.	Upper Asymptote in mm.
28°C.	.77	1.46	1.57	2.82	3.00	4.92
Difference*	.05	.06	.08	.09	.11	.13
23°C.	.82	1.52	1.65	2.91	3.11	5.05
Difference	.03	.05	0.9	.12	.11	.12
18°C.	.85	1.57	1.74	3.03	3.22	5.17

As is seen from the table, differences between asymptotic values for successive growth cycles at different temperatures were all in the same direction. The significancy of their differences can not be proved directly but it may be recognized if we use the probable errors of the actual measurements at the corresponding phases of equilibrium (Table 1) for comparison.

Growth ratios shown for successive lower asymptotes and also for successive upper asymptotes were fairly constant for different temperatures, with average values of 2.03, and 1.88 for lower asymptotes, and 1.92 and 1.73 for upper asymptotes. Here is also seen the gradual decrease of the rate in the later stages as was observed in mouth armature.

4. From the observations on growth ratio of larval length as well as of length of mouth armature, it may be concluded that though these characters attain different sizes at varying temperatures, growth ratio itself is not modified by temperature. Putting it another way, differences of size at varying temperatures increase as the larval growth proceeds but their ratios to the absolute size remain in the same order. Namely, the intensity of thermal effect is observed to be of the same order through the various periods of growth. This fact can be visualized from Table 7 in which the intensity of thermal effect is expressed by the percentage increase of larval length at 23°C. against that at 28°C. and also at 18°C. For larval length, it was on the average 4.1% at 23°C. and 7.9% at 18°C. For mouth armature it was on the average 1.9% at 23°C. and 4.1% at 18°C.

5. Results of measurements of pupae are shown in Table 5. Puparium formation accompanied the shortening of body length but it came to

TABLE 5.  
*Measurement of pupae*

28°C.			23 C.			18°C.		
Time in hrs.	No.	Length in mm.	Time in hrs.	No.	Length in mm.	Time in hrs.	No.	Length in mm.
75	2	3.34	101	5	3.15	168	1	3.82
80	3	3.09	112	16	3.25±.02	180	9	3.23±.02
85	27	3.05±.06	120	5	3.23±.02	192	13	3.34±.02
90	37	3.16±.08	128	18	3.22±.01	204	45	3.32±.01
95	25	3.18±.09	136	20	3.15±.02	228	49	3.28±.01
102	16	3.12±.08	147	16	3.13±.02	264	43	3.32±.01
118	20	3.14±.09	168	22	3.24±.02	276	23	3.34±.02
			174	21	3.25±.02	312	25	3.33±.02
			213	23	3.19±.02	336	22	3.28±.01
			245	16	3.22±.02	360	26	3.33±.02

equilibrium in a short time and remained constant for the rest of the stage. The frequency distribution of pupal length showed two modes and this fact was considered as an indication of sexual dimorphism. However, because of the difficulty in distinguishing sexes, calculations were made for the two sexes together. Biometric constants for the total sample are summarized in Table 6. Results showed thermal effects of the same nature as were seen in the larva, and their differences were statistically

TABLE 6.  
*Biometric constants of pupae*

Temperature	No.	Length of Pupa in mm.	Standard Deviation	Coefficient of Variation
28°C.	126	3.14±.008	.13	4.13±.20
Difference		.07±.012		
23°C.	100	3.21±.007	.12	3.85±.16
Difference		.10±.008		
18°C.	296	3.31±.004	.12	3.49±.11

significant. Percentage decreases in length during puparium formation were 36.2%, 36.6% and 36.0% for the respective temperatures. Therefore the reduction ratio in length in pupation is considered to be independent of temperature.

#### DISCUSSION ON THE RESULTS

It has been demonstrated in the present study that temperature influences the dimensional character of *Drosophila* during the periods of growth and development in the same direction as is already known in the case of the adult form and the egg. It can now, therefore, be stated that thermal effects are revealed at every phase of the life cycles of *Drosophila*. In order to visualize the intensity of thermal effect for the different characters, Table 7 was prepared. In the table there are shown also, for comparison, the intensities already known for eggs and organs of adult forms. The intensity was expressed in the percentage increase of dimension at 23°C. and 18°C. against that at 28°C. Probable error for the figures was calculated from the formula  $\frac{m_1}{m_2} \sqrt{\frac{P.E._1^2}{m_1^2} + \frac{P.E._2^2}{m_2^2}}$ , where  $m_1$  and  $m_2$  are the means of the character at varying temperatures, and  $P.E._1$  and  $P.E._2$  are the probable errors of respective means.



TABLE 7.  
*Intensity of thermal effect*

Characters	Percentage increase at 23°C. against 28°C.	Percentage increase at 18°C. against 28°C.
Egg Length (IMAI, 1934)	4.27±.06%	10.27±.08%
Larval Length; at hatching	6.17±.74%	9.88±.92
Larval Length;		
Stage I { Lower asymptote	6.49%	10.39%
{ Upper asymptote	4.11%	7.53%
Stage II { Lower asymptote	5.10%	10.80%
{ Upper asymptote	3.19%	7.45%
Stage III { Lower asymptote	3.67%	7.33%
{ Upper asymptote	2.64%	5.08%
Average	4.09%	7.88%
Length of Mouth Armature { Stage I	2.35±.21%	5.28±.20%
{ Stage II	1.08±.27%	3.66±.24%
{ Stage III	2.22±.16%	4.12±.29%
Average	1.88%	4.40%
Length of Pupa	2.25±.34%	5.62±.31%
Adult Form		
Wing Length (IMAI, 1933) { ♂	10.99±.05%	21.69±.05%
{ ♀	9.92±.05%	17.20±.05%
Wing Breadth (IMAI, 1933) { ♂	10.20±.05%	18.70±.05%
{ ♀	8.49±.05%	14.43±.05%
Femur Length (IMAI, 1933) { ♂	2.62±.04%	1.29±.05%
First Leg { ♀	4.09±.05%	5.99±.05%
Femur Length (IMAI, 1933) { ♂	3.14±.04%	6.33±.05%
Second Leg { ♀	4.54±.05%	7.99±.05%
Width of Head (ALPATOV & PEARL, 1929) { ♂		3.08±.37%
{ ♀		7.44±.38%
Tibia (ALPATOV & PEARL, 1929) { ♂		7.38±.33%
{ ♀		6.36±.31%

1. There are observed certain variations among intensities at different cycles of growth. Strictly speaking, they show a tendency to decrease gradually as they approach pupal stage. However, these asymptotic values are simply approximate so that no definite conclusion will be possible at present. On the other hand, from the fact that variations were small, it can be stated that the intensities of thermal effects during larval growth were of the same general order. Same thing can be stated as to the dimensions of mouth armature in which the intensities of thermal effect were of the same magnitude for different stage. It should be remembered

here that such a statement is only another way of expressing the fact which has already been spoken of in connection with growth ratio in the preceding chapter.

From these considerations it may be concluded that growth under varying temperatures shows an elevation or decline of the plan of the growth system as a whole. In other words, thermal effects appear as early as at the start of larval life, and remain in the same order of intensities through the various periods of growth. There is no sign whatever that thermal effects accumulate as growth proceeds. This point will be discussed again in connection with the explanation of the nature of the thermal effects.

2. Intensities of thermal effects for different characters such as larval length and length of mouth armature, and also for different stages such as, egg, larva and pupa, show certain variations. Such diversity may be taken as an indication that thermal effect is specific for characters and also for different stages. But such a statement can not be made definitely at present because of doubtfulness in regard to the significance of differences.

On the other hand, it may be stated possibly that through the stages of growth and development, namely, egg, larva and pupa, the intensity of thermal effect is of the same general order, with the range of 2-5% for 23°C. and 4-10% for 18°C.

3. The intensities shown during the periods of larval and pupal stage and those of the organs of adult form cannot be directly related because of the complexity involved in the growth of the organs of adult form. But comparison between them certainly finds meaning in the fact that the latter undergo their growth and development within the system of the former, and there is no doubt that they are under the general control of the former.

There are observed variations among different organs and also between sexes with significant differences as already discussed in 1933. Then how are these intensities for the organs of adult form numerically related to those of larva and pupa? Comparison reveals that, generally speaking, in those characters such as head, femur and tibia, intensities are inside the range observed for the stages of larva and pupa, while in wing dimensions they are extremely high. Though such observation is based on only few characters of adult forms, the fact may be taken to show that in adult form high variation is observed among the intensities of thermal effects on organs with a range from the orders observed during

larval stage to those of two to three times that size.

For the explanation of such high diversities among the organs of adult form we are compelled to consider the specific susceptibility of each organ to temperature during its growth and development as is generally considered. However, in view of the fact that the bud formation and integration of the organs of adult form proceed at two diverse phases, namely at the larval and pupa stages (CHEN, 1929), further experimental analysis seems to be required to see if such extremely high intensities are due only to the specific susceptibility or whether there are other factors which increase the intensities. This is particularly felt under the present conditions of experimental analysis in which it is practically ignored to control humidity inside of the culture bottle. Evaporation of water, which varies with temperature and surrounding humidity, undoubtedly has an influence on the integrative process of organs. It is well known, for numerical characters such as facet number (ZELENY, 1923), and for gene expression such as vestigial (STANLEY, 1931; HARNLY, 1930) and bar-eye (DRIVER, E., 1926; DRIVER, O., 1931; LUCE, 1931), that there are temperature effective periods during the larval stage, but it is the author's view that in dimensional characters such as wing or leg, they are influenced by environmental conditions at both periods of bud formation and integration.

4. Thermal effects on bodily dimensions are of wide occurrence in the animal kingdom and, with few exceptions, it is generally observed that, inside the range of temperature where the animal can grow normally, the lower temperature results in increase of dimensions. A general summary and a bibliography are given by BĚLEHRÁDEK (1935). Most of these studies, however, concern the final size of growth, and few detailed observations have been reported in respect to the periods of growth and development.

As BĚLEHRÁDEK states, there are two diverse hypotheses in regard to the explanation of thermal effect on body size. One considers that it is dependent on the nutritional condition, or that the diminution of dimensional character at the higher temperature is the result of a deficiency of food. The other, however, considers that this is due to the modification of the chemical equilibrium.

We will consider the case presented in the larval growth of *Drosophila* in connection with this problem. Three groups of flies from the same homogeneous strain were kept under three different temperatures 28°C., 23°C. and 18°C., and the growth of their progenies were studied under

strictly controlled conditions. Results showed that at lower temperature there were elevations of the equilibrium points of growth curves at three cycles, and the intensities were found to be of the same general order through the course of growth.

Quality or quantity of food provided does not seem to be an important factor for dimensional change in this case. In our experimental set-up, enough food was provided through the various periods of growth. If there was a deficiency in the amount of food, that could happen during the later part of the growth period. This was evidently not the case, as the thermal effects observed were of the same general order through all the cycles of growth. From such considerations it may be stated that the thermal effects observed here are due to the direct effect of temperature on the physiological processes involved in the growth of the organism.

Starvation experiments reported by ALPATOV (1929) and SMIRNOV (1929) on the dimensional characters of adult form and by GAUSE (1930) on egg size showed an analogy between the effects of high temperature and starvation. From these facts it was suggested that the diminution of size at high temperature is a case of partial starvation. This view must be criticised from the point of the present observation.

The fact that at low temperature the final size is larger than at high temperature, evidently means that more food was consumed for body construction at the lower temperatures than at the higher temperature. But this does not necessarily mean the same thing for the total amount of food consumed during growth. It is generally understood that at higher temperature relatively more energy is expended for maintaining life than for the construction of body, as compared to lower temperatures. For example, GRAY's work on fish (1928), TITCHAK's on moth (1925) and the author's on pond snail (1937) show this point, and the same thing can be expected also in the case of *Drosophila*.

If this view is accepted, it becomes doubtful whether *Drosophila* larva consumes more food during larval growth at lower temperature, and the starvation theory seems to be explained from the modification of the system of metabolic balance during growth under the influence of temperature. From these considerations as well as from the fact that thermal effects appear already at the start of larval life, and that effects with intensities of the same general order appear in the following stages of growth, it is strongly suggested that the thermal effects are connected with the modification of metabolic balance of the growing system.

## SUMMARY

1. Thermal effects on dimensional characters of larval and pupa were studied under controlled environmental conditions.
2. It was proved that temperature influences the larval length, length of mouth armature and pupal length in the same direction as was observed in the egg and the organs of adult form. Namely, high temperature caused the diminution of these dimensional characters.
3. Thermal effects were observed as early as at hatching and effects of the same order were seen in the asymptotic values of successive growth cycles.
4. Dimension of mouth armature was less influenced than the larva as a whole. Intensities were found to be of the same order for the three instars.
5. The pupa was slightly less influenced than the larva.
6. Comparison of the intensities of thermal effects for various stages, egg, larva and pupa, revealed small variations, therefore they may be considered to be of the same general order.
7. High variation in the intensities of thermal effect on the dimensional characters of the adult form was considered to be due to a specific susceptibility of each organ during its growth and development and also possibly to the diversity of phases at which the organogenetic process is subjected to the effects of environmental conditions.
8. The nature of the thermal effects on dimensional character was considered, and it was suggested that the metabolic balance between anabolism and katabolism under the influence of temperature is likely a cause of morphological differences.

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# THE LARVAL SHELL GROWTH OF *LYMNAEA JAPONICA* JAY. IN SPECIAL REFERENCE TO THE INFLUENCE OF TEMPERATURE<sup>1)2)</sup>

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(With four figures)

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## INTRODUCTION

The pond snail, *Lymnaea*, has been a favourite material for experimental work. Since the classic works by J. HOGG (1854) and C. SEMPER (1879), many studies have been made in regard to the morphological changes due to environmental conditions. For study on the growth of the adult form, we can cite several works such as CRABB (1929), BAILY (1931) and NOMURA (1927). In spite of these massive works no experimental work has been made, so far as I am aware, on the larval growth of the animal. The pond snail seems to be a favourite material for study on these points: that it can be reared easily under laboratory conditions, and that its growth and development can be easily observed under the microscope through the egg-membrane and jelly cover which is transparent. Furthermore its shell provides us a concrete structure which can be measured as an index of the growth of the whole organism. The present study was planned with the purpose of studying quantitatively the shell growth during larval life under controlled environmental conditions.

It is widely proved and accepted that higher temperature accelerates the development and growth of animals as it accelerates other biological phenomena except at extremely high temperatures where biological processes may be retarded or disturbed. Beside velocity acceleration, it has been demonstrated in many cases that temperature modifies the final

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size of growth. The characteristics of thermal effect so far demonstrated are not the same in every case, but generally speaking, higher temperature causes diminution of the final size. A general summary and bibliography was given by BĚLEHRÁDEK (1935). However, experiments or observations on this subject have been made mostly on the final size of growth and little is known as to the thermal effects during the stages of growth and development.

The author (1937) studied this problem in the fruit fly, *Drosophila melanogaster* and came to the conclusion that the thermal effect is likely due to the metabolic balance of anabolism and katabolism, in view of the fact that thermal effects appear at every phase of the growth cycles with the same order of intensities. However, at present no definite conclusion will be possible and many more data will be required to understand the nature of the thermal effects on dimensional characters.

The present study will deal with the same problem in the larval shell growth of *Lymnaea*. The system of growth in *Lymnaea* is simplified as compared to the case of *Drosophila* in that larval growth and development come to completion with the given amount of food.

#### MATERIALS AND METHODS

Materials used in this study are the common pond snail, *Lymnaea japonica* Jay., collected in the vicinity of Sendai. They were brought to the laboratory and reared individually in battery jars with filtered aquarium water, and fed with lettuce leaves.

Eggs used in the experiment were those laid by the progenies of the original material which were brought up individually under controlled environmental condition with the temperature at 23°C. Freshly laid eggs were divided into three groups by cutting the clutches with a knife and put in small Petri-dishes with filtered aquarium water. Dishes were put in three electric thermostats with the temperature of 18°C., 23°C. and 28°C. These temperatures were considered to be inside the range where the animals can grow normally. They evidently meet much lower temperatures, particularly in the cold season, but it is doubtful if they grow at such low temperature. In the laboratory conditions with the temperature range above mentioned they grow and lay eggs throughout the year.

As the egg membrane and jelly cover are transparent, it was possible to observe the development and measure shell growth without any further treatment. But in this method certain error of measurements was

unavoidable. Therefore, experiments were also made with separated eggs. In both cases the eggs were numbered and their development and growth were recorded individually. Measurements were carried on with Zeiss microscope with the help of a micrometer eyepiece. The character measured was the total length of shell.

#### TIME RELATION OF THE LARVAL DEVELOPMENT

Like other Pulmonates (C. DAWYDOFF, 1928), *Lymnaea* pass through trochophore, veliger and adult-like form stages before they hatch out of the eggs. Time relations of the successive stages of development under various temperatures are shown in Table 1.

TABLE 1.  
*Influence of temperature on the rate of development.*

Stage	Incubation time at which stage begins.		
	28°C.	23°C.	18°C.
	hrs.	hrs.	hrs.
Trochophore	35- 40	55- 60	65- 70
Veliger	60- 65	90- 95	120-130
Adult-like form	130-140	190-200	260-270
Hatching	195-205	290-300	390 410

As the transformation from one stage to another can not be marked distinctly, the time shown in above table is simply a rough approximation. But it can be stated generally that, inside the range of temperatures here used, the temperature influenced the rate of development in such a way that the duration of incubation is twice lengthened by a decrease of temperature of 10 C. This is true not only for the total duration but also for the duration of each component stage.

In the following Table 2 and figure 1 an example of larval development at 18°C. will be illustrated and some few characteristics observed will be described. The trochophore stage begins at about 70 hours of incubation.

As is clear from these illustrations, the stages with which we are chiefly concerned here in the study of shell growth are those from the veliger stage on. The shell is seen clearly at the early veliger stage. There is no doubt that the shell began development long before this period, possibly in the early trochophore stage. However, practically it is not possible to measure it before this time. Transformation from veliger

TABLE 2.  
*Time relation of the larval development, at 18°C.*

Number	Time of incubation	Larval stage	Characters observed	Length of shell
	hrs.			mm.
1	120	Later trocho-phore stage.	Rotates continuously.	
2	150	Veliger stage	" "	
3	168	" "	Shell is clearly observed and measurable.	0.20
4	195	" "	Still rotates but slowly. The heart beats and the eye spots appear. Foot increases in size but differentiation is not complete. Animal moves as a whole.	0.34
5	215	" "	Still rotates.	0.44
6	226	" "	Still rotates. Foot shows local movement.	0.47
7	240	" "	Rotation is not continuous.	0.50
8	252	Adult-like form.	Crawling begins. Animal moves as a whole. No differential movement of foot.	0.53
9	264.30	" "	Crawling on the egg membrane. Foot moves independently from the rest of the body.	0.57
10	288	" "	Crawling movements are vigorous. Radule movement begins but is not frequent.	0.66
11	312	" "	Radule movements are frequent.	0.71
12	336	" "	" " " "	0.76
13	360	" "	" " " "	0.79
14	385	" "	Begins to break the egg membrane.	0.81

They hatched out of the eggs at about 390-400 hours of incubation.

form into adult-like form is not clear cut. But it is distinguished by the growth and differentiation of the foot and movement of the animal. In the veliger stage the foot has but little function in locomotion. Only the rotation of the animal as a whole is observed. As development proceeds the foot increases in size and is gradually differentiated in its movement. At the stage of adult-like form, crawling motions of the foot are predominantly observed. This crawling movement resembles that which is observed in the adult form. Movements of the lips and also radula appear later in this stage. When they appear, they are infrequent and weak, but their frequency increases gradually and becomes vigorous. Finally the egg membrane is broken and the animal hatches out of the egg in adult form. Even after it comes out of the egg it is still surrounded by the jelly cover. It takes some time before it comes to the outer world.

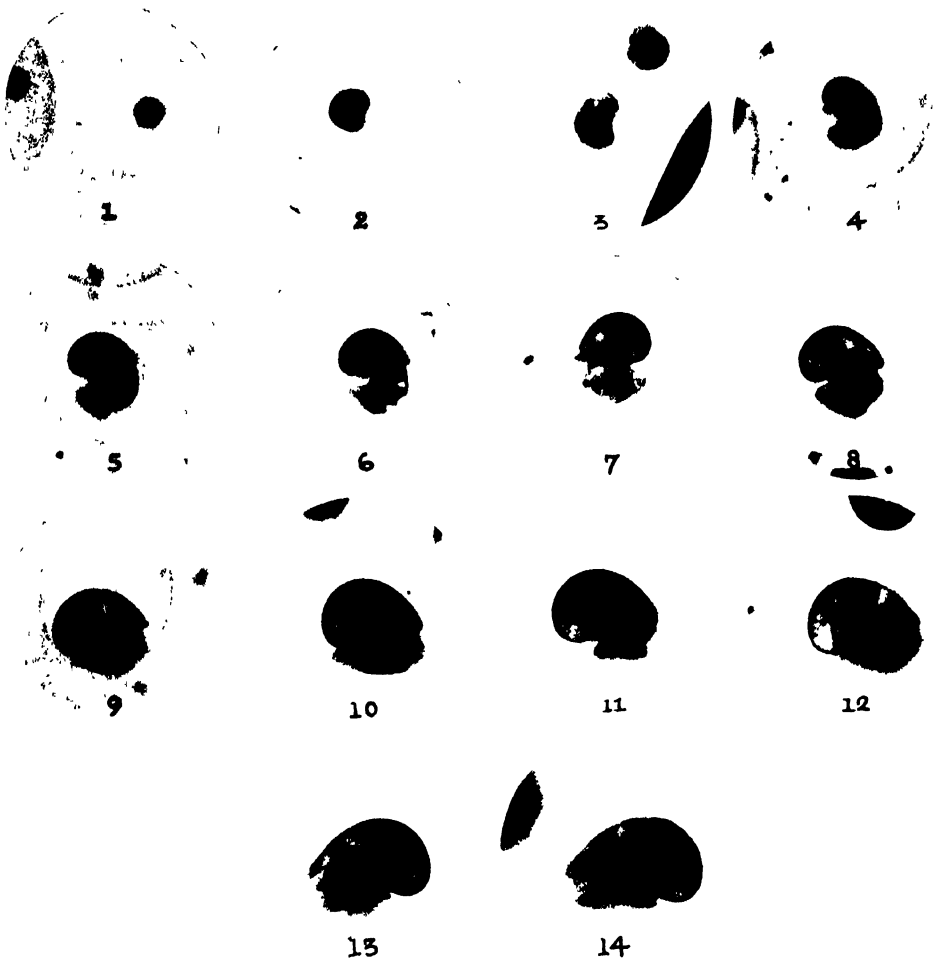


Fig 1 Representation of larval development at 18°C For explanation see Table 2

#### MODE OF SHELL GROWTH

In order to demonstrate the mode of shell growth, examples of growth curves are shown in figure 2 and Table 3. This is the result of Experiment No. 1. As the variations among individuals are small, the description of growth changes is given in terms of averages.

It will be noticed at first glance that the shell growth in the egg is two-fold. Two cycles of growth are observed. The first cycle presumably

begins at size zero and its rate of growth increases gradually, then falls and comes to the resting state at a size of nearly 0.5 mm. Another cycle of growth follows it until the larvae hatch out. The curve of the first cycle shows a logistic character, without any exception so far as studied. The duration of slow growth at the end of the first cycle was found to be fairly uniform in the series where the eggs were kept together in the jelly mass (Exp. No. 1 & 2.), while in the other series where they were separated high variation is observed among individuals (Exp. No. 3). Examples of two extreme cases are shown in figure 3.

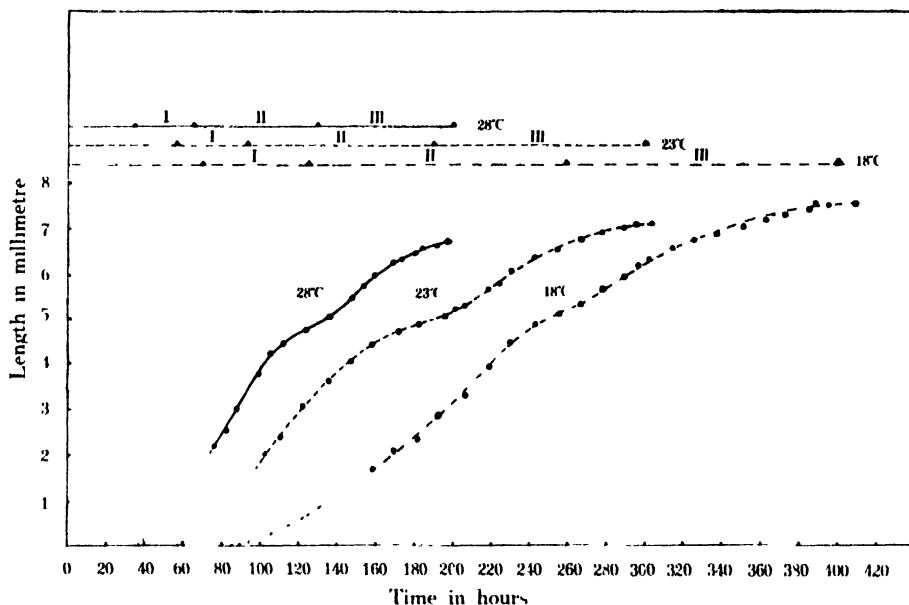


Fig. 2. Duration of the larval stages and growth curves of the larval shells at 28°C., 23°C. and 18°C.

I — Trochophore stage. II — Veliger stage. III — Adult-like form stage.  
— for 28°C. --- for 23°C. . . . for 18°C.

As is clearly seen from the figure, one case shows a resting stage which lasts more than thirty hours (I). During this period no shell growth is observed but later the shell begins to grow again and hatches out of the egg with the same sizes as other individuals that hatched out long before it. In the other case (II), however, the retardation of the growth rate at the end of first cycle lasts for so short a time that it is a little hard to detect.

The form of the growth curve of the second cycle is not so uniform as for the first cycle. In most cases, however, it shows a logistic nature

TABLE 3.  
*Larval shell growth.*  
Experiment No. 1.

23°C. (6 individuals)			23°C. (6 individuals)			18°C. (4 individuals)		
Time of incuba- tion	Average shell length	Number hatched	Time of incuba- tion	Average shell length	Number hatched	Time of incuba- tion	Average shell length	Number Hatched
hrs.	mm.		hrs.	mm.		hrs.	mm.	
75	.219±.0024		102	.203±.0010		153	.172	
81	.256±.0032		110	.235±.0015		158	.177	
87	.304±.0013		123	.308±.0013		170	.203±.0011	
99	.380±.0012		135	.363±.0013		173	.227±.0025	
105	.425±.0012		146	.407±.0010		182	.232±.0008	
111	.446±.0015		158	.446±.0005		192	.293±.0017	
123	.477±.0017		171	.471±.0013		206	.330±.0032	
135	.511±.0022		182	.484±.0020		218	.397±.0040	
147	.550±.0018		195	.512±.0025		231	.447±.0045	
153	.578±.0015		201	.523±.0024		243	.486±.0024	
159	.600±.0013		206	.531±.0010		255	.514±.0013	
169	.620±.0027		217	.550±.0020		267	.540±.0024	
173	.632±.0024		219	.572±.0018		278	.585±.0020	
181	.646±.0027		224	.580±.0020		290	.600±.0039	
184	.654±.0034		231	.609±.0022		296	.622±.0022	
191	.664±.0024	1	243	.639±.0018		302	.632±.0020	
195	.672±.0018	3	255	.652±.0025		315	.661±.0024	
197	.676	2	267	.676±.0022		327	.672±.0020	
			278	.689±.0025		338	.681±.0020	
			290	.698±.0025	2	352	.699±.0037	
			295	.701±.0027	2	363	.716±.0045	
			304	.711	2	368	.733±.0034	
						373	.736±.0039	
						386	.745±.0032	
						389	.757±.0024	1
						396	.750±.0042	
						411	.757±.0051	3

as is shown in figure 2: the growth begins with increasing rate, then gradually decreases and finally comes to equilibrium before the animal hatches out.

Figure 4 shows an example of an irregular form of growth curve that is frequently observed. In this case (I) the second growth cycle is again two-fold and after one rise of the growth curve is over another is observed just before hatching. This mode of shell growth will be considered later in connection with the mechanism of hatching.

We will consider, then, how the mode of shell growth is related to the larval development described in the preceding chapter.

There seems to be no doubt that the retardation of the rate of growth at the end of the first cycle corresponds to the phase of metamorphosis from the veliger stage to the adult-like form. Toward this period the

rotation becomes weaker and discontinuous and the animal gradually begins crawling on the egg membrane with foot which has increased in size and differentiated in movement. By the end of this stage the animal looks like the adult form in its appearance as well as in its mode of movement. From such observations it is considered that the growth of the shell is retarded at the phase of metamorphosis from the veliger stage to the adult-like form. It is not clear why such retardation occurs but it

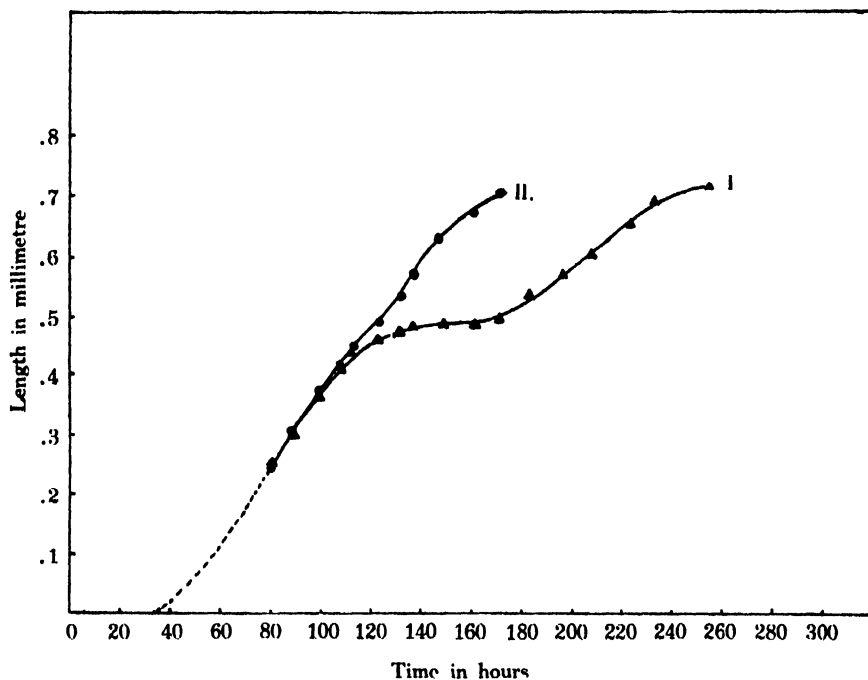


Fig. 3. Examples of the larval shell growth at 28°C. Experiment No. 3. For explanation see text.

may possibly be due to the low metabolic rate. This is suggested from the fact that at this phase the activity of the animal as a whole is seemingly low and the animal is often found staying quiescent.

As soon as the metamorphosis is over the animal begins a second cycle of growth consuming the reserve food left in the egg. The movement of lips and radule — the characteristic mode of feeding in the adult animal — is not observed at the beginning of this stage. The lip movement appears at the stage of shell size of about 0.60 mm. while that of the radule begins still later. The movement of the radule is evidently a

scratch action against the egg membrane. At first it is weak and infrequent but gradually increases its vigour and frequency. Finally the egg membrane is broken and the animal comes out.

From these observations it is suggested that the hatching is due to the mechanical action of the radula. In order to confirm this point the following experiment was performed. Eggs having larva, near hatching, with the shell length of 0.65 mm. were divided into two groups. In both groups the larvae had shown the scratch action of the radule before the experiment was started. A few drops of 0.2% chloreton were added to one group. In this experimental group, the larvae became quiet and no

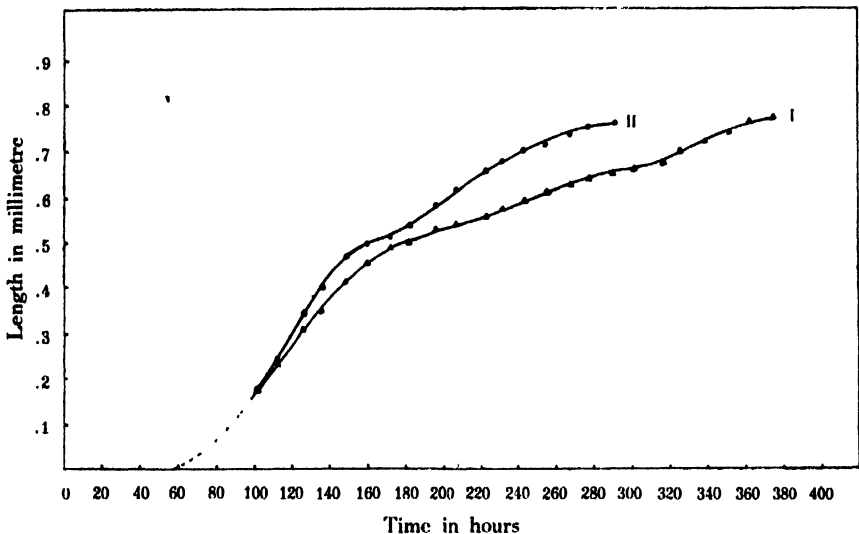


Fig. 4. Examples of the larval shell growth at 23 C. Experiment No. 3. For explanation see text.

scratch action was observed. The result showed that as long as the chloreton was effective the radule movement was restrained and no hatching occurred.

Then how is this radule action initiated? Undoubtedly it is resulted from the development of the radula. But its activity as a hatching agent, seems to be connected with the exhaustion of reserved food inside the egg. At the time when the scratching action of the radula begins, the shell growth approaches the equilibrium phase. This fact indicates that only a small part of the reserve food is left in the egg. It happened in some cases that the hatching is prevented or delayed for a considerable



time and the shell shows no more growth. Furthermore, it is observed that the longer the hatching is delayed the weaker becomes the action of the radula and in a few extreme cases the animal fails to hatch. Consequently it may be concluded that, before hatching, the reserve food inside the egg has been used up or nearly so.

This view will be further supported by the results of the following experiment, that was made to see whether the shell size at hatching was correlated with the volume of eggs. 55 eggs from a single clutch were separated and their growth and development were observed individually until they hatched out. As the egg was ellipsoid, its volume was calculated by measuring the dimensions of two axes. As a result, the correlation table shown in Table 4 was obtained. The coefficient of correlation is  $.40 \pm .08$  and this seems to prove that, in the eggs where a presumably larger amount of food is reserved, the animal reaches larger size before hatching.

In view of the fact that the action of radule lasts for some time before hatching, it may be considered that by this means the inner layer of egg membrane itself is consumed as food. This view seems to be supported by the fact that the third cycle of growth is often distinguished just before hatching as is shown in (I) of figure 4.

TABLE 4.

*Correlation between egg volume and shell length at hatching.*

	Egg Volume (mm. <sup>3</sup> )									Total
	1.45- 1.49.	1.50- 1.54.	1.55- 1.59.	1.60- 1.64.	1.65- 1.69.	1.70- 1.74.	1.75- 1.79.	1.80- 1.84.	1.85- 1.89.	
Shell length at hatching, mm.										
.73			2							2
.74	1		2	1	1					5
.75		2			1	1				4
.76		1	2	2		1				6
.77				4	1					5
.78		1	1	1	1	1				5
.79		1	3	2		2	1			9
.80		1			1		1	1	1	5
.81		1	2	1	1	1	2			8
.82				1	1					2
.83							1			1
.84					1			1		2
.85			1							1
Total	1	7	13	12	8	6	5	1	2	55

The general scheme of shell growth and the mechanism of hatching in *Lymnaea* may be summarized in the following way. Larval shell

growth begins as early as at the trochophore stage, and through the veliger stage it completes one cycle following a logistic pattern. Here the larvae metamorphose to adult-like form and while the metamorphosis proceeds shell growth is delayed. Another cycle of growth proceeds by consuming the food substances left in the egg. As the reserve food comes near to exhaustion the growth is gradually retarded again. At this stage the hatching action, i.e. the movement of the radule, begins and it gradually increases in vigorousness till the egg membrane is finally broken and the larva finds its way out.

#### INFLUENCE OF TEMPERATURE ON SHELL GROWTH

In view of the fact that there are two cycles of shell growth during larval life, we may consider the thermal effects at two phases. In the first place it will be asked how the size at hatching is influenced by temperature, and in the second how the size at the equilibrium phase of the first growth cycle is influenced by temperature. Results of three sets of experiments are summarized in Table V. Values for the asymptotic

TABLE 5.

*Influence of temperature on shell length.*

Experiment number	Size of egg	Temperature	Number	Shell length at the end of the first growth cycle	Shell length at hatching
I	Major axis	28°C.	6	mm.	mm.
	0.9024 $\pm$ .0062	23°C.	6	.4909 $\pm$ .0026	.6735 $\pm$ .0021
	Minor axis	18°C.	4	.5056 $\pm$ .0032	.7055 $\pm$ .0023
	0.6832 $\pm$ .0033			.5329 $\pm$ .0017	.7503 $\pm$ .0036
II	Not measured	28°C.	6	.4984 $\pm$ .0023	.6752 $\pm$ .0029
		23°C.	6	.5214 $\pm$ .0041	.7245 $\pm$ .0020
		18°C.	6	.5325 $\pm$ .0014	.7516 $\pm$ .0027
III	Major axis	28°C.	6	.4813 $\pm$ .0016	.6959 $\pm$ .0050
	0.9161 $\pm$ .0045	23°C.	5	.5198 $\pm$ .0016	.7447 $\pm$ .0028
	Minor axis	18°C.	6	.5309 $\pm$ .0025	.7603 $\pm$ .0021
	0.6758 $\pm$ .0014				

phase at the end of the first cycle of growth are estimated individually by fitting a curve free hand. Therefore it cannot be more than a simple approximation. The values for the size at hatching are by actual measurement. The growth curves obtained in the experiment No. 1 has already been shown in figure 2.

So far as the mode of shell growth is concerned, no difference is observed in the different temperature series. However, the sizes at hatching are influenced by temperature as is seen from both the tables and the figures. That is to say, the lower the temperature of incubation the larger becomes the size of shell at hatching. This same fact can be observed in the shell size at the end of the first growth cycle, though the figures are only approximately given. From these facts it may be concluded that, under the influence of temperature during incubation, *Lymnaea* larvae grow in such a way that smaller size is attained at a higher temperature as compared to lower temperature during both the veliger and the adult-like form stages.

#### DISCUSSION OF THE RESULTS

In this study an attempt has been made to find the mode of shell growth during larval life in *Lymnaea*. Another attempt has also been made to see how temperature influences the growth of the shell. The system of growth we consider here is such that there is a determined amount of food provided for the growth. The results of observations show that the shell is formed at the trochophore stage and increases its size through both the veliger and adult-like form stages until finally it hatches. The mode of growth during these stages is not a simple function and two cycles of growth are observed: one cycle up to the end of the veliger stage, and the other during the adult-like form stage.

From observations on the development of the animal and also on the hatching mechanism, the appearance of two equilibrium phases will be explained as follows. The equilibrium phase at the end of the veliger stage is brought on as a result of development and differentiation of the animal. That is, at this phase the growth rate decreases because of the metamorphosis. On the other hand, the retardation of growth at the hatching phase may be considered due to the exhaustion of the amount of food reserve, as already observed.

The observations on growth under various temperature conditions show that lower temperature of incubation causes not only increase of the shell size at hatching, but also an elevation of equilibrium size at the end of the veliger stage. Thermal effect observed at the phase of hatching seems to indicate that, with a given amount of reserve food to begin with, the metabolic efficiency during larval growth is modified by temperature. That is to say, the animal grows and develops rapidly at higher temperature but this is carried on at the expense of a larger amount of energy

than at lower temperature. This fact agrees with GRAY's results on the embryonic growth of trout (1926) and can be taken as an indication that metabolic balance between anabolism and katabolism is modified by temperature during growth periods.

From the thermal effects observed at the phase of equilibrium of the first cycle of growth, it can be stated that at higher temperature the animal metamorphoses at smaller size than at lower temperature. Considering that there is plenty of reserve food left for further growth, this fact must be explained from the thermal effect on the physiological process itself involved in growth and development. In view of the fact that the metabolic balance during the growth period is modified by temperature, it is strongly suggested that it is such basic influence of temperature on metabolic balance which results in the dimensional change at the phase of metamorphosis.

#### SUMMARY

1. Growth of the larval shell of *Lymnaea japonica* JAY. was studied under controlled temperature conditions.
2. Growth of the larval shell is two-fold. The first cycle comes to a state of rest at the stage of metamorphosis from veliger to adult-like form. The second cycle consists of the growth during the adult-like form stage. At the end of this growth hatching occurs.
3. The first cycle of growth shows a logistic character. The second cycle also has the same nature in most cases, though certain irregularities are often found.
4. There is an indication that hatching occurs when the reserve food is exhausted or nearly so.
5. Hatching is due to the mechanical action of the radula.
6. The growth under varying temperature conditions shows that higher temperature result in the acceleration of the development but the final size is diminished at both the first cycle of growth and the second one.
7. As to the nature of thermal effect on shell size, present results seem to indicate that it is fundamentally due to the modification of the metabolic balance in the growing system.

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# ON THE COLLECTION OF BRYOZOA ALONG THE COAST OF ONAGAWA BAY AND ITS VICINITY, THE NORTHERN PART OF HONSHŪ, JAPAN

By

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(With Plate XI and five text-figures)

(Received March 8, 1937)

The present report is based on the collection of Bryozoa obtained along the coast of Onagawa Bay last summer by Mr. T. IMAI and Mr. H. SATŌ, the members of the Onagawa Oceano-chemical Institute of the Tōhoku Imperial University and submitted to us for identification by Prof. S. HŌZAWA. There are 19 species in the collection, of which 4 species seem to be new to science. We take this opportunity of expressing our sincere thanks to Prof. S. HŌZAWA, Mr. T. IMAI and Mr. H. SATŌ for their kindness in contributing valuable materials for our studies.

Order CHEILOSTOMATA BUSK

Suborder ANASCA LEVINSSEN 1909

Division MALACOSTEGA LEVINSSEN 1909

Family EUCRATIIDAE HINCKS 1880

Genus *Eucratea* LAMOUROUX 1812

## 1. *Eucratea chelata* (LINNAEUS) 1758.

*Sertularia chelata* LINNAEUS, 1758, Syst. Nat., ed. 10, p. 816

*Eucratea chelata* LAMOUROUX, 1810, Mem. Class. Poly. Corall., p. 64, pl. 3, fig. 5.—  
BUSK, 1880, Chall. Rep., p. 3—CANU & BASSLER, 1929, U. S. N. M., Bull. 103, vol. 9, p. 58,  
fig. 1, H; fig. 2, A-g.—OKADA, 1934, Sc. Rep. T. B. D., Sect. B, vol. 2, no. 26, p. 2.

**Diagnosis:** The zooecia in the form of a horn are sub-calcareous, rising immediately one from the other so as to form a single series. The zooecial aperture is oval, oblique; the peristome is thin, a little raised, and the membranous part of frontal depressed. The branches given off from the front of a zooecium directly below the aperture. The oecia are endozooecial, mitriform, acuminate, subcalinate.

**Distribution:** Cosmopolitan. Japan—Akane, Shimoda, Shizuoka-ken.

**Locality:** An incomplete colony attached to *Crisia bucinaform* OKADA was obtained at a spot in Koyatori, 14 m. in depth (Sp. no. 749e).

**Note:** The present specimen has rather elongated zooecia but no ooecia.

Family BIFLUSTRIDAE SMITT 1872

Genus *Conopeum* NORMAN 1903

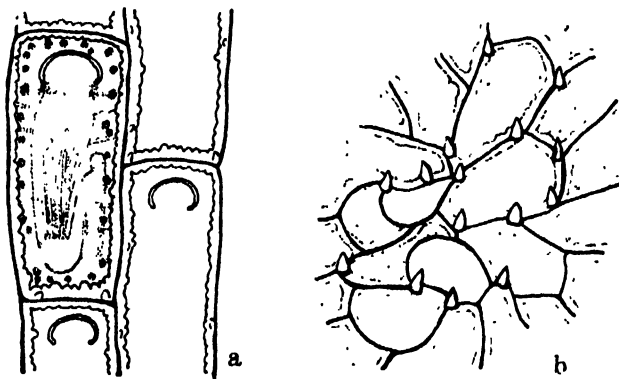
2. *Conopeum serrata* (HINCKS) 1882.

(Pl. XI, fig. 11; Text-fig. 1)

The synonyms and diagnosis are cited in Y. OKADA's paper on the Bryozoa of Mutsu Bay, 1929, p. 11.

**Locality:** Several complete colonies, forming circular patches on seaweeds were collected at Igonohama 8.5 m. in depth (Sp. no. 618).

**Note:** The specimen before us somewhat remarkable in the zooecial features. The crenations of the zooecial walls rather resembles that of



Text-fig. 1. *Conopeum serrata* (HINCKS).

- a. A few zooecia one of them with polypide inside.
- b. Zooecia near the ancestrula with distinct spinous denticles on the ventral surface.

*Membranipora serrulata* BUSK than that of *Conopeum serrata* (HINCKS). A few of the long distinct denticles, found frequently at irregular intervals on the zooecial walls are described and figured by A. ROBERTSON and Y. OKADA, but in the present specimen these denticles are usually not developed, especially in young zooecium. But some zooecia with very indistinct denticles, and the older zooecia near the ancestrula have the prominent denticles at each distal angle.

## Division CELLULARINA SMITT 1867

## Family BUGULIDAE GRAY 1848

Genus *Bugula* OKEN 18153. *Bugula neritina* (LINNAEUS) 1758.

Cited in the paper on the Bryozoa of Mutsu Bay, p. 13.

**Localities:** Many complete colonies with or without ooecium were obtained on the shore of Ishihama (Sp. no. 406), the shore of Nakano-shima (Sp. no. 469b), the shore of Tsukahama (Sp. no. 992), at Izushima (Sp. no. 1118a), and at Konorihama (Sp. no. 1234b).

4. *Bugula dentata* (LAMOUROUX) 1816.

*Acamarchis dentata* LAMOUROUX, 1816, Hist. Poly. Corall., p. 135, pl. 3, figs. 3a, b.

*Acamarchis tridentata* KRAUSS, 1813, Beit. Kent. Corall. Zoophy. Südsee, p. 31, pl. 3, figs. 2a-c.

*Bugula dentata* BUSK, 1852, Cat. Mar. Poly. Brit. Mus., pt. 1, p. 46, pl. 35, figs. 1-5.—MACGILLIVRAY, 1883, Prod. Zool. Vict., 8, p. 30, pl. 78, figs. 3, 3a.—ORTMANN, 1890, Arch. f. Naturgesch., 56, 1, p. 25, pl. 1, fig. 20.—KIRKPATRICK, 1890, Sci. Proc. Dublin Soc. (N.S.), 6, p. 614.—WATERS, 1897, Journ. Linn. Soc. Zool., 34, p. 5, pl. 1, fig. 1.—CALVET, 1905, Rev. Suisse Zool., p. 617, pl. 21, fig. 1.—NORMAN, 1909, Journ. Linn. Soc. Zool., p. 285, pl. 36, fig. 3.—LEVINSEN, 1909, Publ. Carlsberg Fund., p. 101, pl. 5, figs. 1a-b.—THORNELEY, 1912, Trans. Linn. Soc. Zool., p. 141.—YANAGI & OKADA, 1918, Annot. Zool. Jap., vol. 9, pt. 4, p. 421.—MARCUS, 1922, Abhandle. Senckenberg Naturf. Ges., p. 422, pl. 24, fig. 1.—O'DONOGHUE, 1924, Rep. Univ. S. Africa, Fish & Mar. Biol. Surv., 3, p. 33.—HARMER, 1926, Siboga Exped., 28, b, pp. 439-441, pl. 30, figs. 5, 6; pl. 32, figs. 21-25. OKADA, 1934, Sc. Rep. T. B. D., Sect. B, vol. 2, no. 26, p. 5, pl. 1, fig. 9.

**Diagnosis:** The zoarium is erect, forming a bushy tuft, not originated from a stalk formed of kenozoecia; the branches biserial, narrow, of uniform width. The zooecia are distinct, elongate, rectangular; the edges hardly projecting; the external margins rolled inward and the frontal membrane more or less oblique. The opecia occupying most of the frontal surface, narrower proximally. There are 1 inner and 2-3 outer spines on the distal margin of the zooecia, generally long and jointed at the base. The avicularia are of two kinds, the ordinary ones elongate on the outer side of the opecia, and the larger ones with long, shallow, weak rostra and triforiate mandibles sometimes on the two zooecia at a bifurcation, and less commonly on the other parts. The ooecia are large, globose, hyperstomial, directed obliquely inwards. The rootlets are relatively coarse.

**Distribution:** S. Africa; Cape Verde Isl.; Madeira; Australian Sea; Japan—Moroiso, Jôgashima, Yokohama, Kanagawa-ken; Miyakejima, Izu-



shichitô; Shimoda, Shizuoka-ken; Tomo, Okayama-ken; Kushimoto, Wakayama-ken.

**Locality:** Several colonies with ooecia were obtained at Konorihama attached to the oyster (Sp. no. 1234a).

### Family SCRUPOCELLARIIDAE LEVINSEN 1909

#### Genus *Scrupocellaria* VAN BENEDEN 1845

#### 5. *Scrupocellaria macandrei* BUSK 1852

*Scrupocellaria macandrei* BUSK, 1852, Cat. Brit. Mar. Poly., 1, p. 24, pl. 24, figs. 1-3.—BUSK, 1861, Quart. Journ. Mic. Sc., 1, p. 77.—BUSK, 1884, Chall. Rep., vol. 10, pt. 30, p. 23.—HELLER, 1867, Bry. Adr., p. 87.—PHILLIPS, 1899, Will. Zool. Res., pt. 4, p. 442.—CALVERT, 1906, Exp. Sc. Trav. Talism., pt. 8, p. 375.—WATERS, 1913, Proc. Zool. Soc., p. 477, pl. 98, figs. 5, 6.—YANAGI and OKADA, 1918, Annot. Zool. Jap., vol. 9, pt. 4, p. 415.

**Diagnosis:** The zoarium forming a coarse bushy mass 15 mm. in height, dichotomously branching; the internodes consisting of 9-17 zooecia. Joints are conspicuous, occurring as high on the zooecium as the proximal margin of the aperture. The zooecia are arranged alternately and biserially, distinct, ovate above, slightly narrowed below; the aperture is oval, occupying about half the front; the scutum is very large, entire, reniform, expanded below, covering almost whole the aperture. There are usually 5 oral spines on the upper one-third of the apertural margin, 3 on the outer and 2 on the inner. The peculiar granular area exists around the lower half of the aperture, broader below, the granules are arranged in somewhat radiating rows. The lateral avicularia are as large as the aperture, conspicuous with strongly hooked beak, always found on each zooecium; the frontal avicularia none. The vibracular chamber is dorsal, situated just below the lateral avicularium. its length equalling half that of the zooecium; vibraculum long running dorsally and frontally. The rootlet arises from near the base of vibracular chamber, developing only on the lower zooecia of a zoarium. Unfortunately the specimen before us has no ooecia.

**Distribution:** Cape Verde Isl.; Spain; Adriatic; Queensland; Zanzibar; Japan—Odawara, Jôgashima, Kanagawa-ken; Senzaki, Yamaguchi-ken; Tsushima, Nagasaki-ken.

**Locality:** Several complete colonies were obtained at a spot off Oura Inlet, 8 m. in depth (Sp. no. 912a).

**Note:** The present specimen is closely allied to *Scrupocellaria scrupea* BUSK, but differs from it in having the larger scutum, and in the presence of granular margin of the zooecial aperture.

Genus *Caberea* LAMOUROUX 18166. *Caberea hataii* OKADA 1929

*Caberea hataii* OKADA, 1929, Sc. Rep. Tôhoku Imp. Univ., Biol., vol. 4, no. 1, fasc. 1, p. 13, pl. 1, fig. 2, pl. 4, fig. 2, text-fig. 2.

**Localities:** Several small colonies were obtained at a spot of Izushima Harbour, 28 m. in depth (Sp. no. 594), and a western spot of Takojima, 31 m. in depth (Sp. no. 723).

Genus *Menipea* LAMOUROUX 18167. *Menipea occidentalis* TRASK 1857

*Menipea occidentalis* TRASK, 1857, Proc. Cal. Acad. Sc., p. 113, pl. 4, fig. 4.—ROBERTSON, 1905, Univ. Calif. Publ. Zool., vol. 2, p. 254, pl. 6, figs. 22-25.—YANAGI & OKADA, 1918, Annot. Zool. Jap., vol. 9, pt. 4, p. 409.

*Menipea compacta* HINCKS, 1882, Ann. Mag. Nat. Hist., 5, vol. 10, p. 461.—HINCKS, 1884, Ann. Mag. Nat. Hist., 5, vol. 13, p. 208, pl. 9, fig. 8.—ORTMANN, 1890, Arch. f. Naturgesch., p. 21, pl. 1, fig. 2.

**Diagnosis:** The zoarium forming a bushy tuft attached by numerous root fibres. The branching is regular, dichotomous; the internodes consist of three or more zooecia. The zooecia are elongate, narrowed below; the aperture occupying about half the front surrounded by usually 6 jointed spines, 2 on each lateral margin of the upper half of the aperture and 2 others on distal margin. The scutum is not wide forming a mere spine or rod, sometimes a spoon or scalpel, usually entire but not rarely bifid or more divided. The lateral avicularia are large, occurred on each zooecium except the one at the bifurcation of the branch; the frontal avicularia are wanting. The ooecia are hyperstomial, large, globose, perforated by a small number of pores. The rootlets extend downwards arising from the lower side of the root chambers just above the lateral avicularia of the lower zooecia.

**Distribution:** San Francisco Bay; Queen Charlotte Isl.; San Diego; Japan—Misaki, Yokohama, Kanagawa-ken; Ôzu, Ibaragi-ken; Saseho, Nagasaki-ken; Shinojima, Aichi-ken; Hakodate, Hokkaidô.

**Locality:** Several fragments were obtained on the shore at Miyagasaki (Sp. no. 1037b).

**Note:** The present specimen differs from the Californian species of ROBERTSON in the number of the zooecia of an internode and in the shape of the scutum. From these characters the present specimen shows rather an intermediate type between *Menipea occidentalis* TRASK and its variety *catalinensis* ROBERTSON.

### 8. *Menipea occidentalis* var *catalinensis* ROBERTSON 1905

*Menipea occidentalis* var. *catalinensis* ROBERTSON, 1905, Univ. Calif. Pub. Zool., vol. 2, no. 5, p. 255, pl. 7, figs. 26, 27.—OKADA, 1918, Annot. Zool. Jap., vol. 9, pt. 4, p. 409.—OKADA, 1929, Sc. Rep. Tōhoku Imp. Univ., Biol., vol. 4, no. 1, fasc. 1, p. 15, pl. 1, fig. 3.

Diagnosis is cited in OKADA's paper of 1929.

**Distribution:** Santa Catalina, San Pedro, California; Japan — Hakodate, Hokkaidō; Takanoshima, Chiba-ken; Misaki, Ehime-ken; Shimoda, Shizuoka-ken; Mutsu Bay, Aomori-ken.

**Localities:** Several colonies and fragments were obtained at Ishihama, shore (Sp. no. 404); Nakanoshima, shore (Sp. no. 468); at Takenoura, shore (Sp. no. 1272) and at a spot of Ommae Inlet, 12 m. in depth (Sp. no. 574a).

**Note:** The present specimens quite agree with the description of ROBERTSON. The features of scutum are more or less variable but there seems some difference between this variety and the typical species.

### Suborder *Ascophora* LEVINSEN 1909

### Family *ESCHARELLIDAE* LEVINSEN 1909

### Subfamily *Schizoporellae* CANU & BASSLER 1917

### Genus *Dakaria* JULLIEN 1903

### 9. *Dakaria typica*, n. sp.

(Pl. XI, fig. 6, Text-fig. 2)

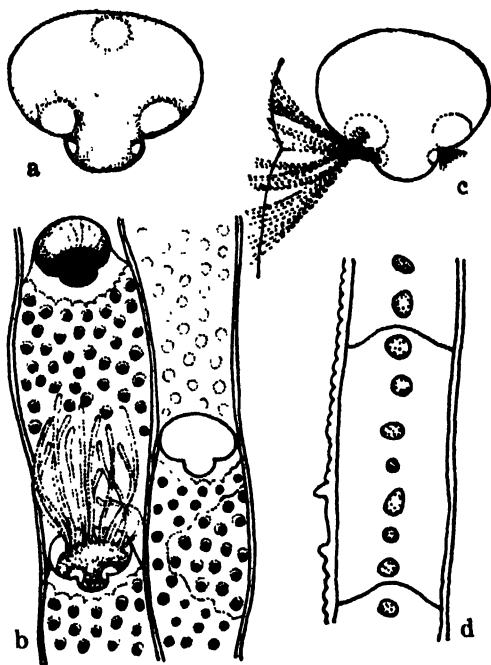
**Diagnosis:** The zoarium is rather thick, lobate, sometimes undulate, uni- or bi-lamellar, incrusting or crustaceous unattached. The zooecia are distinct, separated by salient threads, rectangular or elongate elliptical, arranged in radiating linear series. The frontal is large pored tremocyst, little convex, smooth, sometimes minutely granulated in the older zooecia; the tremopores with elevated margin; the primary calcification takes place at first on the proximal part and lateral walls of zooecia and progresses distally. The colour of the frontal membrane is brown in alcohol, lighter in the younger zooecia and darker in the matured one; but later on the frontal membrane is striped off while the secondary calcification is completed, then the frontal turns grayish white, opaque. The aperture is circular or elliptical, with large rounded rimule proximally; the fringe arises somewhat from the zooecial plane, making a low, thin peristome. The non-perforated area surrounding the peristome is broader or narrower. The operculum is circular or elliptical with large rounded projection on

the middle of the lower margin, dark brown almost black, with a fainter circular area on each side of the lower margin. The operculum articulates on two small condyles of the aperture characteristic in *Dakaria*. The basal side of the zooecium is smooth, thin, separated by furrows. The ooecia are not found. The avicularia none. The lateral wall has 5-7 multiporous septula arranged longitudinally; the septule is circular or elliptical and the middle one is usually the smallest; the number of minute pores in each septule varies greatly, the range being 3-20. The varying number of scattered multiporous septula of different form and size occur in the distal wall.

**Localities:** Several colonies were obtained at Ishi-

hama, shore (Sp. no. 405); at Nakanoshima, shore (Sp. no. 467); at Sannōjima, shore (Sp. no. 958); and at Konorihama (Sp. no. 1234c).

**Note:** The present specimen is very closely allied to *Dakaria bidentata* (ORTMANN) but differs from it in having a circular aperture with a rounded rimule and in the smaller sometimes indistinct condyles as well as in the form of the operculum. This also resembles *Schizoporella oenochros* ORTMANN, but differs from it in having wide rimule and tremopores with elevated margin, as well as salient threads surrounding the zooecia.



Text-fig. 2. *Dakaria typica* n. sp.

- a. Operculum with three thinner circular areas.
- b. A few zooecia to show the zooecial arrangement; the polypide extruding the tentacles from the aperture.
- c. Operculum with muscle bundles.
- d. Multiporous septula of the lateral wall of zooecium.

Subfamily *Microporellae* CANU & BASSLER 1917Genus *Microporella* HINCKS 187710. *Microporella ciliata* (PALLAS) 1766

The synonyms and descriptions being referred to the paper on the Bryozoa of Mutsu Bay, pp. 26-27.

**Localities:** Two complete colonies attached to sea-weeds were obtained at a spot near Koshikine, 25 m. in depth (Sp. no. 713e) and at a spot of Ommae Inlet, 10 m. in depth (Sp. no. 930).

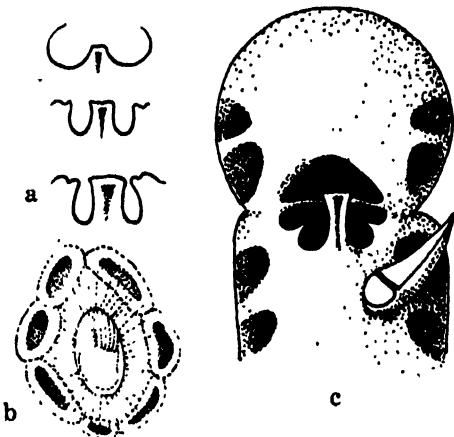
**Note:** The specimens before us have prominently projected ooecia with granulate surface.

Subfamily *Peristomellae* CANU & BASSLER 1917Genus *Exochella* JULLIEN 188811. *Exochella areolata*, n. sp.

(Pl. XI, figs. 3-5, Text-fig. 3.)

**Diagnosis:** The zoarium incrusts sea-weeds, forming a circular patch, thin and unilamellar. The zooecia arranged radially are distinct, surrounded by deep furrows, short, hexagonal, trapezoid; the frontal is convex sur-

rounded by very large areolar pores of small number. The peristome is salient bearing 3 distal spines in the younger state. The oral mucro is salient, rather large, foliated, erect, hiding a part of the zooecial aperture; there are also 2 distinct denticles of elevated margin of the peristome on each side of the mucro, samely hiding the aperture. The avicularium is large situated on one side of the peristome a little below the oral mucro, with elongated triangular mandible, more or less curved and pointed at the end. The ooecium is hyperstomial, very large, globose, with smooth



Text-fig. 3. *Exochella areolata* n. sp.

- a. Variations of lyrula.
- b. Dorsal surface of the zooecium to show the membrane and polypide.
- c. Zooecium with ooecium.

frontal surface surrounded by peculiar areolar pores, halfly imbedded in the distal zooecium, and is separated by a distinct boundary from the aperture. The basal surface of the coecia are quite membranous bordered by the thick walls with dietella.

**Locality:** A small but complete colony was obtained at Koshikine, 19 m. in depth (Sp. no. 706).

**Note:** The present species is closely related to *Exochella lobata* LEVINSSEN but differs from it in having 3 spines and having the larger areolar pores of smaller number surrounding the zooecia and ooecia.

### Family SMITTINIDAE LEVINSSEN 1909

#### Genus *Smittina* NORMAN 1903

#### 12. *Smittina landsborovii* (JOHNSTON) 1849

*Lepralia landsborovii* JOHNSTON, 1849, Brit. Zooph., 2, p. 310, pl. 54, fig. 9. BUSK, 1852, Cat. Mar. Poly. Brit. Mus., 2, p. 66, pl. 86, fig. 1; pl. 102, fig. 1.—HINCKS, Quart. Journ. Micr. Sc., 8, p. 226.

*Smittina landsborovii* SMITT, 1867, Oefv. k. Vet. Ak. Forh. Bihang, pp. 12, 92, pl. 26, fig. 63.—OKADA, 1923, Annot. Zool. Jap., 10, 22, p. 228.—OKADA & MAWATARI, 1936, Sc. Rep. T. B. D., Sect. B, no. 49, p. 65, pl. 10, fig. 8, text-fig. 4.

**Diagnosis:** The zoarium incrusting, unilamellar, thin, milky white in alcohol. The zooecia are arranged alternately in many radial lines from the ancestrula, distinct, elliptical, subhexagonal, somewhat<sup>4</sup>elongate, separated by furrows. The frontal is slightly convex, formed by a pleurocyst bordered by many remarkable areolar pores with salient costules. The peristome is thin, elevated; the orifice is circular, lunar, or oval, more or less transverse, with a prominent rimule in the middle of the lower margin; the cardelles indistinct; the lyrula moderate. The median frontal avicularium situated just below the rimule, has semicircular or lip-like mandibles and prominent pivot. The surrounding area of the peristome and avicularium is somewhat elevated, smooth or more minutely granulated. The ooecium is hyperstomial, smooth, perforated, imbedded in the distal zooecium.

**Distribution:** Northumberland; Shetland; Durban; Natal; Florida; Greenland; Japan—Hamajima, Mie-ken; Chôshi, Chiba-ken; Kurokutô, Yamagata-ken; Itô, Shizuoka-ken.

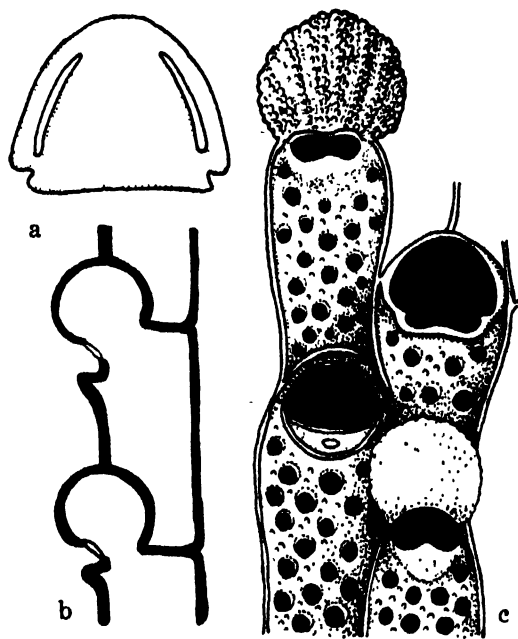
**Note:** The present specimens quite agree with the description and figures of HINCKS, 1880.

**Locality:** A complete but small colony was obtained at a spot near Yatarôjima, 34 m. in depth (Sp. no. 902).

Genus *Mucronella* HINCKS 188013. *Mucronella perforata*, n. sp.

(Pl. XI, fig. 8, Text-fig. 4.)

**Diagnosis:** The zoarium is lobate, crustaceous, undulate, uni- or bi-lamellar, attaching to sea-weeds. The zooecia are arranged alternately in many linear series, distinct, surrounded by salient threads, elliptical or oval; the frontal is convex, finely perforated by rather large pores. The aperture is large, semicircular with two small condyles on both sides of the proximal margin. The operculum is semicircular with broad frontal projection and emphasized by the lateral thickenings. The granulated prominent oral mucro situating just below the aperture projecting forwards with flat horizontal upper plane. There is a peculiar pore of the compensation sac in the middle of the plane. The avicularium none. The oecia are hyperstomial, closed by the opercula, large, globose, thickly granulated in somewhat radial lines. Sometimes abnormal zooecia are found in a zoarium.

Text-fig. 4. *Mucronella perforata* n. sp.

- a. Operculum with two condyles.
- b. Diagrammatic figure of the sections of zooecial chambers.
- c. Zooecia with oecia to show the various forms of the aperture and a peculiar pore of the compensation sac.

**Locality:** One complete colony was obtained at a spot north of Yatarôjima, 34 m. in depth (Sp. no. 899a).

## Family CELLEPORIDAE BUSK 1852

Genus *Costazia* NEVIANT 189514. *Costazia costazii* (AUDOUIN 1826)

(Pl. XI, fig. 7)

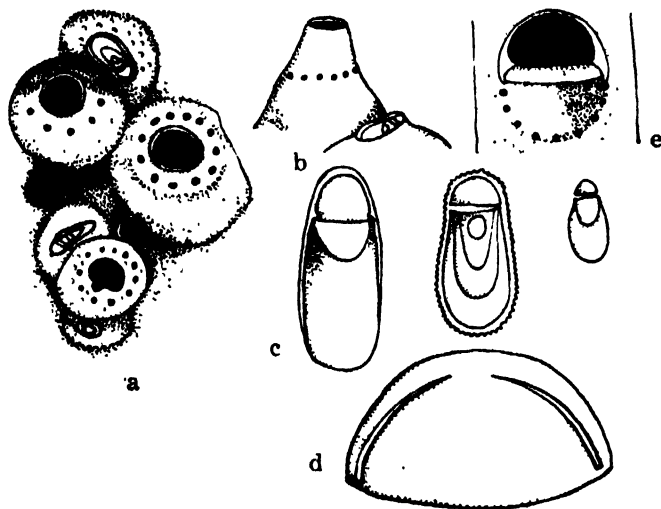
Cited to the report of Mutsu Bay, pp. 33-34.

**Localities:** Numerous colonies were obtained at a spot of Koyatori, 14 m. in depth (Sp. nos. 749c, 949d); at Miyagasaki, 10 m. in depth (Sp. no. 524); at a spot of Oura Inlet, 8 m. (Sp. no. 912b); at Takonoshima, 2 m. (Sp. no. 944); and at a spot of Takenoura Inlet, 15 m. (Sp. no. 1212b).

Genus *Holoporella* WATERS 190915. *Holoporella projecta*, n. sp.

(Pl. XI, fig. 2, Text-fig. 5)

**Diagnosis:** The zoarium is flat, undulate, multilamellar, forming a somewhat thick irregular mass. The normal zooecia are salient, orbicular

Text-fig. 5. *Holoporella projecta* n. sp.

- a. A few zooecia with avicularia.
- b. A projected peristome.
- c. Three kinds of avicularian mandibles.
- d. Operculum.
- e. Zooecial aperture from inside.



or rectangular basally, conspicuously globose with prominently projected peristome; the frontal is very convex, granular and surrounded by a circle of small pores a little distance of the margin. The zooecial aperture is semielliptical, hidcd and is not seen frontally but the circle of the pores indicates the position of the aperture; the peristomial orifice is circular. The ooecia are hyperstomial, and the ovicelled zooecia indicate the true zooecial aperture with low peristome; the operculum is semicircular or somewhat bell-shaped with broad frontal projection, of the typical form of this genus. The avicularia of different sizes are found usually on the globose convexed part of the normal zooecia, just before the circle of the small pores, with spatulate mandibles and pivots and with elevated margin. The deep zooecia have their orifice visible only.

**Locality:** A complete colony was obtained at a spot north of Yatarô-jima, 34 m. in depth (Sp. no. 899b).

Suborder *Hexapogona* CANU & BASSLER 1927

Family MYRIOZOIDAE SMITT 1866

Genus *Myriozoom* DONATI 1750

16. *Myriozoom marionensis irregulatum* OKADA 1923

*Myriozoom marionensis irregulatum* OKADA, 1923, Annot. Zool. Jap., vol. 10, art. 22, p. 231, pl. figs. 27-31.—OKADA, 1923, Sc. Rep. T. B. D., sect. B, vol. 2, no. 26, p. 19.—OKADA & MAWATARI, 1935, Sc. Rep. T. B. D., sect. B, vol. 2, no. 25, p. 142, fig. 5.

**Diagnosis:** The zoarium is continuous, composed of long struggling cylindrical branches of uniform diameter, divercating irregularly, usually at right angles, occasionally anastomosing and constricted at irregular intervals. The zooecia are completely immersed, with no visible outlines, disposed quincuncially on all sides of branches; the surface is perforated round the zooecial aperture. The aperture is completely immersed with a small rimule, looking directly upwards, transversely elliptical; anterior border thin and entire. A small avicularium with a spatulate mandible on each side just within the border of the zooecial aperture. The ooecia are large, rounded above, with a flattened area anteriorly where a series of pores of varying size exists.

**Distribution:** Straits of Korea; Shimoda, Itô, Shizuoka-ken.

**Locality:** Two fragments were obtained at a spot off Yoriiso, 39 m. in depth (Sp. no. 889b, c).

Order *Cyclostomata* BUSKDivision *Inovicellata*

## Family CRISIIDAE JOHNSTON 1847

Genus *Crisia* RAMOUROUX 181217. *Crisia bucinaform* OKADA 1928

*Crisia bucinaform* OKADA, 1928, Sc. Rep. Tōhoku Imp. Univ., Biol., vol. 3, no. 4, fasc. 1, pp. 486-487, pl. 24, fig. 2, text-fig. 4.

**Locality:** A small fragment was obtained at a spot of Oura Inlet, 8 m. in depth (Sp. no. 912b).

Family *Tubuliporidae* JOHNSTON 1836Genus *Tubulipora* LAMARCK 181618. *Tubulipora pulchra* MACGILLIVRAY 1885

(Pl. XI, fig. 9)

op. cit. p. 489, pl. 14, fig. 3; text-figs. 6a-c.

**Locality:** Several colonies attached to sea-weeds were collected at Izusima, shore (Sp. no. 1170).

Family *Heteroporidae* PERGENS & MEUNIER 1886Genus *Heteropora* BLAINVILLE 183019. *Heteropora* sp.

(Pl. XI, fig. 10)

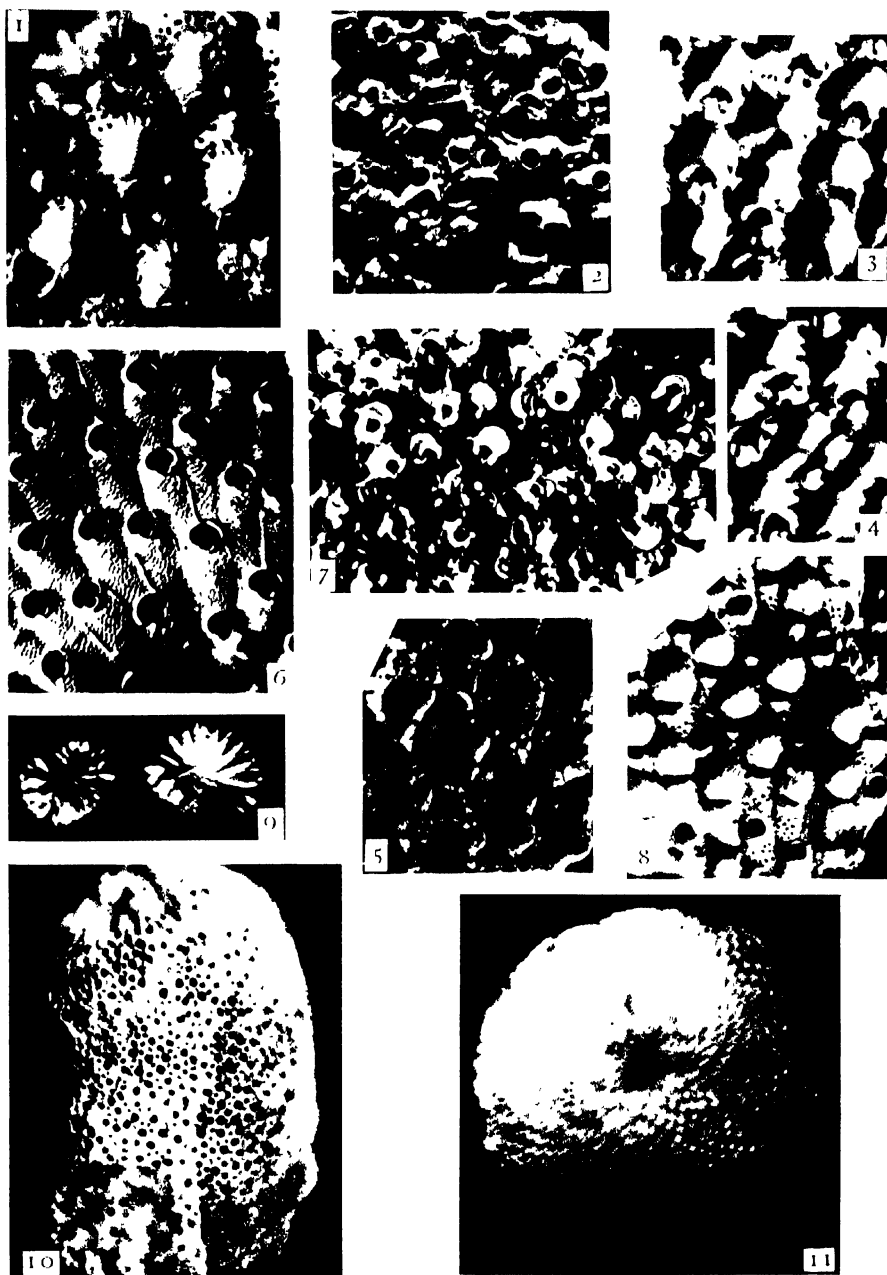
**Locality:** A single fragment was obtained at a spot off Yoriiso, 39 m. in depth.

**Note:** The specific determination should be revised in the future collection.

## EXPLANATION OF PLATE XI.

- Fig. 1. *Microporella cihata* (PALLAS) × 50 (p. 440)
- Fig. 2. *Holoporella projecta* n. sp. × 12 (p. 443)
- Fig. 3. *Exochella areolata* n. sp. × 50 (p. 440)
- Fig. 4. *Exochella areolata* n. sp. × 50 (p. 440)
- Fig. 5. *Exochella areolata* n. sp. The basal surface. × 50 (p. 440)
- Fig. 6. *Dakaria typica* n. sp. × 12 (p. 438)
- Fig. 7. *Costasia costazii* (AUDOUIN) × 12 (p. 443)
- Fig. 8. *Mucronella perforata* n. sp. × 12 (p. 442)
- Fig. 9. *Tubulipora pulchra* MACGILLIVRAY × 6 (p. 445)
- Fig. 10. *Heteropora* sp. × 6 (p. 445)
- Fig. 11. *Conopeum serrata* (HINCKS) × 6 (p. 434)





Y. OKADA AND SH. MAWATARI: Brvozoa of Onagawa Bay.



# STUDIES ON THE GROWTH HORMONES OF PLANTS.

## II. EFFECT OF HETERO-AUXIN ON THE GROWTH OF *HELIANTHUS* HYPOCOTYL

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(With four figures)

(Received March 8, 1937)

### I. INTRODUCTION

CZAJA (1935 a) has shown that a lanoline paste, containing a growth substance in high concentration, inhibits the growth of the hypocotyl of a *Helianthus* seedling, and, at the same time, causes it to swell if applied to it just under the cotyledons of the intact seedling, although this paste causes a further growth and no swelling of the hypocotyl, if applied to the cut surface of a decapitated seedling, or of a seedling not decapitated but split longitudinally between the cotyledons. Judging by the above facts, he supposed that the growth inhibition and the swelling occur because the action of the stream of the normal growth substance, directed downwards in the hypocotyl, is inhibited by the another stream of additional growth substance in a high concentration from outside, and the latter may be directed at right angles to the former. Further he states (CZAJA, 1935 b, c) that two streams of growth substance flowing in opposite directions also counteract each other from which a growth inhibition may result.

Recently, LE FANU (1936) has reported that the growth of young internodes of *Pisum sativum* is inhibited by hetero-auxin lanoline if this is pasted on the stem in a position morphologically below them, although their growth is accelerated by this paste when applied to them from above. Thus she has come to the following conclusion: "The nature of auxin action, whether it is to be acceleration or inhibition, appears to be determined by one thing—the position of the auxin source relative to the organ to be affected." Further she considers that the inhibition of elongation is probably an indirect effect of the growth substance.

In the present work, the writer reports the investigation of the effect of hetero-auxin upon the growth of a *Helianthus* hypocotyl, having reached

an entirely different conclusion as to the cause of the growth inhibition and of the swelling produced by the growth substance.

## II. MATERIAL AND METHODS

Etiolated seedlings of *Helianthus annuus* were used as plant material. After the pericarps had been removed, the seeds of this plant were sown in sawdust, and kept at about 25°C in a dark moist chamber. In most cases, the seeds were sown so that their radicles might point downwards.

The growth substance used was the pure synthetic hetero-auxin<sup>1)</sup> ( $\beta$ -indolyl acetic acid). The hetero-auxin-lanoline paste was prepared after LAIBACH's method (LAIBACH, 1933 b). As the original paste (1/1-paste), distilled water containing 0.8% of hetero-auxin was mixed with an equal amount of anhydrous wool fat. 1/2, 1/4, 1/8-pastes and others were obtained by diluting the 1/1-paste with pure lanoline paste, which is made by mixing an equal amount of water and anhydrous wool fat.

The experiments were made in a dark room. Operation and other treatments were carried out under a red lamp (24 c. p.) covered additionally with two sheets of red paraffin paper. The treated seedlings were planted again in sawdust and were kept at about 25°C in the dark moist chamber during the experiment.

## III. EXPERIMENTS

### 1. Effect of the Hetero-auxin Paste Applied to the Cut Surface of the Cotyledons after Removal of the Upper Halves.

The upper halves of the cotyledons of the seedling are removed, and

TABLE 1.

*Effect of the Hetero-auxin Paste upon the Hypocotyl Elongation.  
Paste Applied to the Cut Surface of Cotyledons.*

Exp. No.	No. of plants	Average length of hypocotyls (mm)					Duration of exp. (hrs.)
		Initial length	Length after experiment				
			1/2-paste	1/4-paste	0-paste	Intact contr.	
74	8	7	—	10.6	13.0	—	17.5
53	7	13	19.3	—	29.5	30.1	22
55	9	40	—	53.1	75.7	73.6	20
59	5	117	135.4	136.2	152.0	151.6	19

<sup>1)</sup> The hetero-auxin, synthesized by Prof. Dr. R. MAJIMA, was obtained through the kindness of Prof. Dr. S. FUJISE to whom the writer wishes to express many thanks.

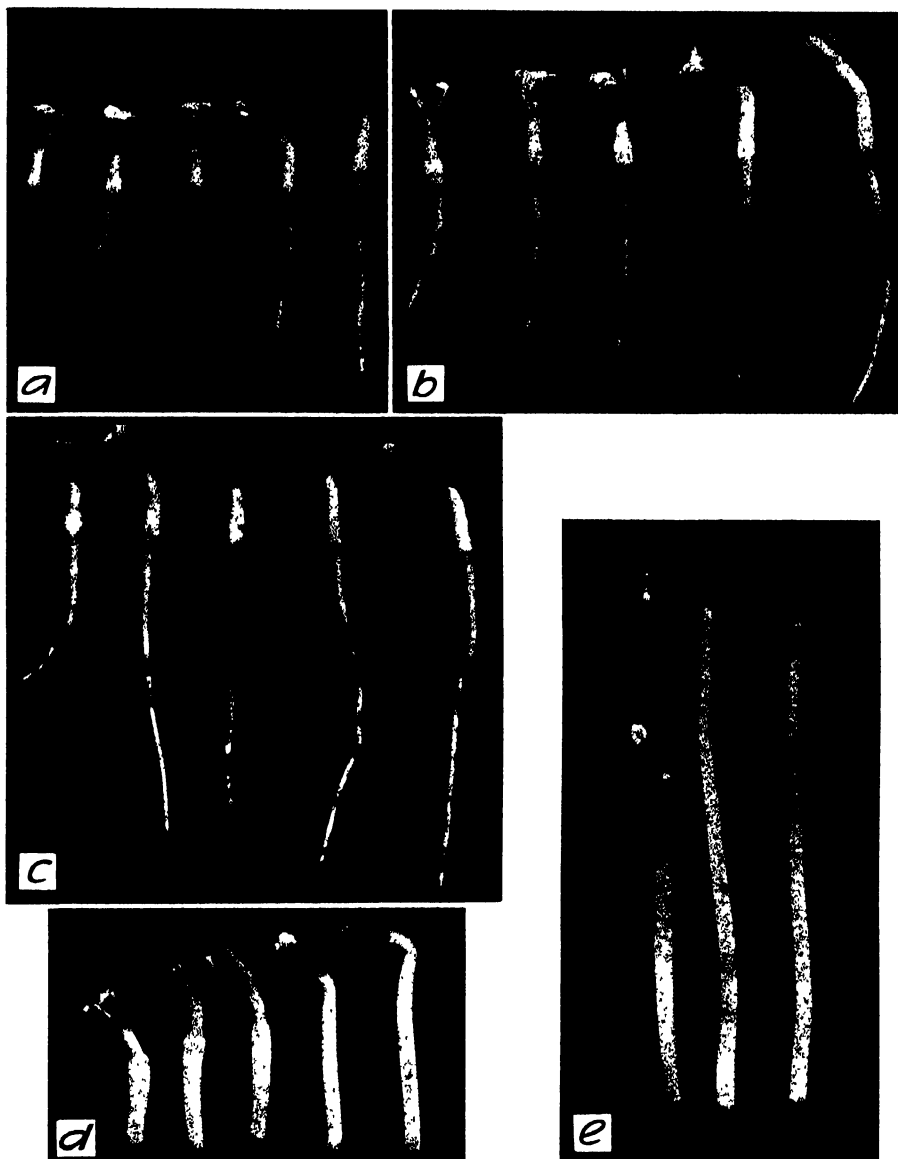


Fig. 1. *Helianthus* seedlings. Upper halves of cotyledons were removed. Heteroauxin paste was applied to the cut surface. Phot. after 17-20 hrs. ( $\times 1$ ) (a) Left to right: 1/1-paste, 1/2-paste, 1/4-paste, 0-paste, intact. Initial length (hypocotyl+root) 9 mm. (b) Pastes are the same as (a). Initial length: hypocotyl 6.5 mm, root 9.5 mm. (c) 1/8-paste, 1/16-paste, 1/32-paste, 0-paste, intact. Initial length: hypocotyl 6 mm, root 11.5 mm. (d) 1/2-paste, 1/4-paste, 1/8-paste, 0-paste, intact. Initial length: hypocotyl 13 mm, root 53 mm. (e) 1/4-paste, 0-paste, intact. Initial length of hypocotyl 37 mm.



the paste is applied to the cut surface. By this method, the growth substance is supplied to the hypocotyl from above, and may be transported in parallel to the plant axis, while the organ to be affected is not injured. After a definite time, the length of the hypocotyl is measured. Results are shown in Tab. 1 and Fig. 1.

The above experiment gives us the following facts:

- i. The elongation of the hypocotyls of plants supplied with hetero-auxin is inhibited.
- ii. A marked swelling arises on the hypocotyls of the treated plants. The swelling starts abruptly several mm below the cotyledons.
- iii. In the treated plants, the apical bending of the hypocotyl does not increase.<sup>1)</sup>

In addition to the above facts, the effect of hetero-auxin on the root elongation must be mentioned. The elongation of this is remarkably inhibited by strong hetero-auxin paste applied to the cut surface of the cotyledons (Tab. 2 and Fig. 1). However, it is not yet decided by this experiment whether such inhibition is caused by the hetero-auxin transported from the cut surface of the cotyledons to the elongating portion of the root or is the indirect effect of the hetero-auxin. This problem of the effect of hetero-auxin upon the root growth, however, will be dealt with in another paper.

TABLE 2.

*Effect of the Hetero-auxin Paste upon the Root Elongation.  
Paste Applied to the Cut Surface of Cotyledons.*

Exp. No.	No. of plants	Initial length (mm)		Root length after experiment (mm)								Duration of exp. (hrs.)
		Hypo-cotyl	Root	1/1-paste	1/2-paste	1/4-paste	1/8-paste	1/16-paste	1/32-paste	0-paste	Intact contr.	
45	13	8.7	11.8	13.8	16.8	—	—	—	—	22.3	26.1	18
47	10	8.5	—	—	—	—	22.4	23.5	27.2	25.9	29.7	19
46	10	6.4	11.2	20.9	25.3	29.8	—	—	—	41.7	43.4	17
48	9	5.9	12.2	—	—	—	34.9	42.3	46.6	48.4	50.9	17
49	5	12.2	45.8	—	64.8	71.4	75.8	—	—	91.4	90.6	18

<sup>1)</sup> When the seeds of *Helianthus* are sown, so that their radicles may point downwards, their hypocotyls grow straight at first, but later begin to bend in the upper portion. The direction of the bending may possibly be determined by gravity in the early stage of development of the seedling (SPERLICH, 1912). In the present experiments, the hypocotyl began to bend two days after sowing (hypocotyl length about 10 mm). The measurement of the length of seedlings showing an apical bending was carried out along the median line between the convex and concave sides.

It is shown by the above experiment, that hetero-auxin paste when applied to the cut surface of the cotyledons inhibits the elongation of the hypocotyl. We have then to determine in what part of the hypocotyl the elongation is inhibited. To solve this problem, an experiment similar to the above was made, using hypocotyls marked with Indian ink at regular intervals. The zones between the marks were numbered in order from upper part to base. Results are shown in Tables 3 and 6 as follows :

TABLE 3.

*Effect of the Hetero-auxin Paste upon the Growth of Different Parts of Hypocotyl. Paste Applied to the Cut Surface of Cotyledons. Initial Length of the Hypocotyl 7 mm.*

Strength of paste	Length of each zone in mm after 17.5 hrs. (Average of 5 plants)						
	I	II	III	IV	V	VI	VII
1/4	3.9			1.3	1.8	1.8	1.5
0	5.3			2.2	2.2	2.1	1.4
							Total
							10.4
							13.3

Swelling of the hypocotyl started from middle part of the zone IV.

In small seedlings, elongation is inhibited in almost all parts of the hypocotyl supplied with hetero-auxin (Tab. 3), while in taller seedlings, the hetero-auxin paste causes growth inhibition in the upper half and a slight acceleration in the lower (Tab. 6). These facts are also shown to be the case by the next experiments.

## 2. Effect of the Hetero-auxin Paste Applied to One Side of the Seedling.

### a) Upper halves of both cotyledons removed, and the paste applied to the cut surface of one of them.

*Exp. with young seedlings* (hypocotyl nearly straight, length 8 mm or less): When a hetero-auxin paste stronger than 1/8 was used, a positive apical bending of the hypocotyl was caused and a swelling appeared on the side to which the paste had been applied (Fig. 2 a). In the lower part of the hypocotyl, no bending occurred. An apical bending occurred also in the seedling treated with the 1/16 or 0-paste, but its direction was indifferent to the position of the paste. In the plant treated with hetero-auxin, elongation was inhibited in every zone except the lowest (Tab. 4).

TABLE 4.  
*Effect of the Hetero-auxin Paste Applied to the Cut Surface of One of the Cotyledons on the Growth of the Different Parts of Hypocotyl. Initial Length of Hypocotyl 6 mm.*

Strength of paste	Length of each zone in mm after 17.5 hrs. (Average of 4 seedlings)						Total
	I	II	III	IV	V	VI	
1/4	1.3	1.3	1.3	1.5	1.7	1.8	8.7
0	1.8	1.6	1.7	2.0	2.1	1.5	10.6

Swelling of the hypocotyl started from middle part of the zone IV.

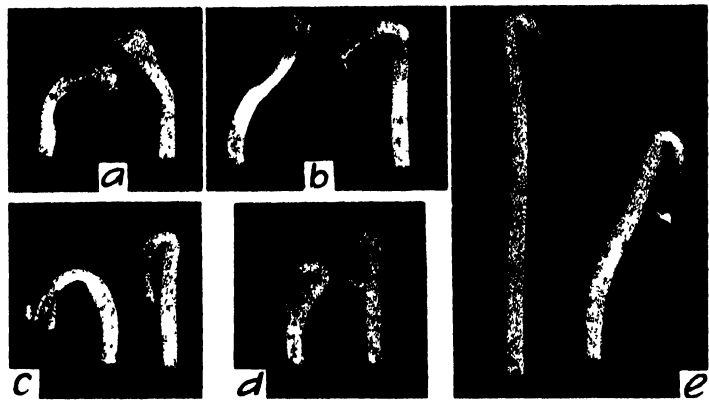


Fig. 2. *Helianthus* seedlings. Upper halves of cotyledons were removed. 1/4-paste was applied to the cut surface of one of the cotyledons. Phot. after 18-24 hrs. ( $\times 1$ ) (a)-(d): right plants are controls, (e): left is control. (a) and (b): seedlings were almost straight before exp. (c) (e): seedlings showed apical bending before exp

- (a) Paste applied to right cotyledon. Initial length of hypocotyl ca. 6 mm.
- (b) " " left " " " " ca. 10 mm.
- (c) " " outer " " " " 12 mm.
- (d) " " inner " " " " 10 mm
- (e) " " outer " " " " ca. 25 mm.

*Exp. with older seedlings* (Lenth of hypocotyl 10-25 mm): Seedlings nearly straight or showing an apical bending in the plane perpendicular to the cotyledonary were used for the experiment. When the hetero-auxin paste (1/4) was applied to the cut surface of one of the cotyledons, a negative curvature of the hypocotyl was caused in the middle or the lower portion of it after three or four hours. Curvature of that grade, as seen in Fig. 2 (b-e), were even reached after about 8 hours. In the

case of the seedlings which showed a marked apical bending of the hypocotyl, the paste was applied to either the outer or the inner cotyledon. No appreciable difference was found in either case. A swelling appeared only on the treated side. But, when the paste was applied to the cut surface of the outer cotyledon of a seedling showing an intense apical bending, the non-treated side also swelled more or less. A swelling seems to begin after several hours and to increase slowly. After 10 hours the swelling was far smaller than that shown in Fig. 2. The apical bending remained almost the same during the experiment in the treated plants, while it increased in the control.

*b) Paste applied to one side of the hypocotyl of the intact seedling.*

*Exp. with the seedlings whose hypocotyl length is 10-12 mm:* A small mass of the paste was applied to the apical, middle or basal part of the hypocotyl. In a hypocotyl having the hetero-auxin paste (1/4) applied at the apical part, negative curvatures began to occur, after about 4 hours, in the middle or in the lower part. When the paste was applied to the middle part of the hypocotyl, a slight negative curvature was caused, in some cases, after 9 hours in the lower part of it. The paste applied to the basal part of the hypocotyl caused no curvatures in the treated plants. Fig 3A shows the results of the experiments after 22 hours. On the other hand, there is also a difference in the mode of swelling in each case: The paste applied to the apical part caused a swelling only on the treated side, but when it was applied to the middle or to the basal part, some swelling occurred also on the opposite side. Moreover, in the latter case, i. e., when the paste was applied to the basal part, the swelling was marked at the base. The apical bending of the hypocotyl did not increase in all seedlings supplied with hetero-auxin.

*Exp. with the seedling whose hypocotyl length is 23-43 mm:* The paste was applied in stripes several mm long, on one side of the hypocotyl, to its upper, middle or lower part. After one hour of experimentation the middle parts of all the hypocotyls having the hetero-auxin paste (1/4) on these or on the upper parts showed negative curvatures. When the paste was applied to the lower part of the hypocotyl it caused, in some cases, also negative curvatures in the part thus treated or somewhat above it. After two hours of experimentation the negative curvatures of all the treated seedlings increased. Results after 20 hours of experiment are shown in Fig. 3B. A slight swelling occurred in the plants treated, but growth was inhibited in all the plants which had been thus supplied

with hetero-auxin. From this experiment, it is clear that the hetero-auxin paste affects the growth of the hypocotyl from the basal portion as well as from the apex.

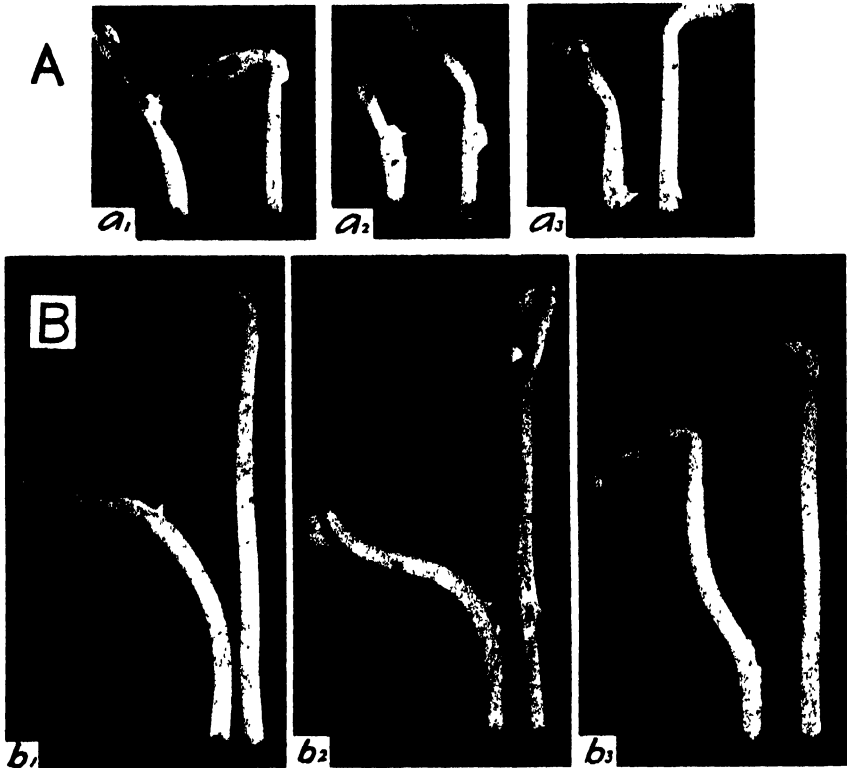


Fig. 3. *Helianthus* seedlings. 1/4-paste was applied to one side of hypocotyl. Phot. one day after. ( $\times 1$ ) Initial length of hypocotyl: (A) 10-12 mm; (B) ca. 30 mm.  $a_1$  and  $b_1$ : paste on upper part;  $a_2$  and  $b_2$ : paste on middle part;  $a_3$  and  $b_3$ : paste on basal part. Right plants are controls.

As was shown by the above experiments, the seedling which was supplied with hetero-auxin to one side of its hypocotyl, as was expected, showed a growth acceleration in the middle or the lower part of that side, giving rise to a negative bending. But this bending extended more or less to that part of the hypocotyl, where the elongation was found to be inhibited at the end of the experiment. In that part, the retardation in elongation appears to occur after the preliminary acceleration. On the other hand, the hypocotyl showed no positive curvature, although the elongation of its upper part was inhibited. This result is probably due

to the fact that the capacity for elongation in the uppermost part of the hypocotyl diminishes generally, not only on the treated but also on the opposite side.

These results show that neither CZAJA's theory (1935) nor LE FANU's (1936) can explain the growth inhibition and swelling of the hypocotyl caused by the hetero-auxin paste in the above experiment, because this paste was supplied in most cases to the hypocotyl from above and in parallel to the organ axis. So that for the cause of growth inhibition in the above experiment the most probable explanation may be found by taking into consideration the excess of growth substance in the respective part. The next experiment supports this view.

### 3. Effect of the Hetero-auxin Paste Applied to the Cut Surface of the Decapitated Hypocotyl.

The seedlings of the same length were decapitated at various heights, and the paste was applied to the cut surfaces. Measurement was carried out after a definite time. Results are shown in Tabs. 5 and 6, and Fig. 4. In these tables, the results of parallel experiments, where the paste is applied to the cut surfaces of cotyledons, are also incorporated.

TABLE 5.

*Effect of the Hetero-auxin Paste upon the Hypocotyl Elongation. Paste Applied to the Cut Surface of Cotyledons or Hypocotyl. Initial Length of Hypocotyl 13 mm. Duration of Exp. 22 Hrs.*

Strength of paste	Intact control	Upper halves of cotyledons removed		Decapitated at ca. 1 mm below cotyledons		Decapitated at ca. 5 mm below cotyledons	
		0	1/2	0	1/2	0	1/2
Average length of 7 hypocotyls (mm)	30.1	29.5	19.3	22.3	15.8	9.9	11.6

As the tables 5 and 6 show, the total elongation of the hypocotyl is inhibited, when the hetero-auxin paste is applied to the cut surface of the cotyledons as well as to that of the hypocotyl decapitated at about 1 mm below the cotyledons. In both cases, the inhibition occurs in the upper part of the hypocotyl, while the elongation of the lower part is accelerated. On the other hand, when the decapitation is done at about 5 mm or more below the cotyledons, the elongation of the hypocotyl is accelerated in every zone by the hetero-auxin paste applied to its cut sur-

TABLE 6.  
*Effect of the Hetero-auxin Paste upon the Hypocotyl Elongation. Paste Applied to the Cut Surface of Cotyledons or Hypocotyl. Hypocotyl Marked at 5 mm Intervals.<sup>1)</sup>*

Exp. No.	No. of plants	Operation	Strength of paste	Length of each zone in mm									Duration of exp. (hrs.)		
				0	I	II	III	IV	V	VI	VII	VIII		IX	Total
58	6	Intact	—	14.3	12.4	8.3	6.0	5.1	5.0					51.1	20
		Upper halves of cotyledons removed	0	15.3	13.3	8.9	6.1	5.1	5.0					53.7	
		Decapitated at ca. 1 mm below cotyledons	0	7.3	9.0	7.5	6.4	5.5	5.1					40.8	
		Decapitated at end of zone II	1/4	14.0	10.5	7.7	5.8	5.3	5.0					48.3	
61	5	Intact	—	10.4	9.3	8.9	7.6	6.2	5.3	5.1	5.1	5.0	5.0	62.9	17
		Upper halves of cotyledons removed	0	10.7	10.2	9.4	7.5	6.1	5.2	5.1	5.0	5.0	5.0	64.2	
		Greater part of cotyledons removed	0	9.9	8.6	8.1	6.9	6.2	5.3	5.1	5.0	5.0	5.0	60.1	
		Decapitated at upper end of zone I	1/4	7.3	6.8	7.2	6.4	5.7	5.2	5.2	5.1	5.0	5.0	53.9	
77	5	Decapitated at end of zone I	0	8.0	9.3	8.5	7.2	6.3	5.3	5.1	5.0	5.0	5.0	59.7	19
		Decapitated at end of zone I	0	—	5.3	6.0	5.9	5.6	5.2	5.1	5.1	5.0	5.0	43.2	
		Decapitated at end of zone I	1/4	—	8.1	7.5	6.6	6.1	5.3	5.1	5.0	5.0	48.7		
		Intact	—	7.1	14.5	11.9	7.8	5.6	5.0					44.8 <sup>2)</sup>	
77	5	Upper halves of cotyledons removed	0	7.2	14.6	11.3	8.2	5.9	5.1					45.1 <sup>2)</sup>	19
		Decapitated at 5 mm below cotyledons	1/4	5.0	7.6	8.6	6.8	5.9	5.2					34.1 <sup>2)</sup>	
		Decapitated at 5 mm below cotyledons	0	—	8.1	7.1	6.3	5.5	5.0					32.0	
		Decapitated at 10 mm below cotyledons	1/4	—	8.2	8.8	7.1	5.9	5.1					35.1	
77	5	Decapitated at 10 mm below cotyledons	0	—	—	5.4	5.3	5.0	5.0					21.3	19
		Decapitated at 10 mm below cotyledons	1/4	—	—	7.7	7.2	6.1	5.0					26.0	

<sup>1)</sup> In the experiments of Nos. 58 and 61, the zone I starts from a turning point of the hypocotyl top, a few mm below the cotyledons. In the experiment of No. 77, the zone I starts from a point 5 mm below the cotyledons.

<sup>2)</sup> Length of the zone 0 is not included.

face. A marked swelling occurred in the hypocotyl the elongation of which was inhibited by the paste. In the case, where the acceleration of hypocotyl elongation was caused by the paste, the swelling was not clearly marked, although the treated plants became generally thicker than the control.

Thus it is established that the same zone may respond in a quite different way to the same hetero-auxin paste, according to the degree of decapitation. This response seems to be regulated by the amount of growth substance in the zone to be affected. When that has a normal growth substance in a sufficient amount for its own growth, it is obvious that elongation is inhibited by a further supply of growth substance, as this would cause an excess of it. On the

other hand, the elongation of the zone is accelerated by the added growth substance, if the amount of normal growth substance in the zone is not sufficient for its own elongation. The fact that after the removal of the upper halves of the cotyledons<sup>1)</sup> the elongation of the upper part of hypocotyls often shows a tendency to accelerate (Tabs. 1 and 6) suggests that, in intact seedlings, that part of the hypocotyls has rather an excess amount of growth substance, — which may come from the cotyledons or the plumule, or may be produced in the hypocotyl (i. e., in the topmost several mm or in the entire growing part) by materials coming from the cotyledons. From this, it follows that when the hypocotyl is decapitated, the stump is deprived of the supply of normal growth substance or materials to produce it and a shortage of this substance may be caused in it.

The other way of explanation, however, may not entirely be excluded from the probability in this case, as the growth inhibition above mentioned

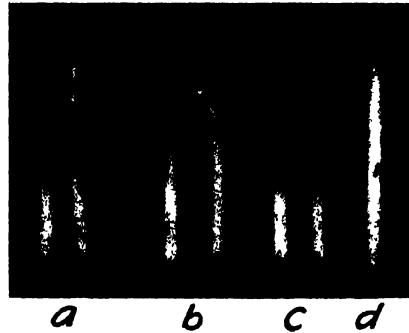


Fig. 4. *Helianthus* seedlings. 1/2-paste was applied to the cut surface. Initial length of hypocotyl 13 mm. Phot. after 23 hrs ( $\times 1$ ) (a) Upper halves of cotyledons were removed. (b) Decapitated at ca. 1 mm below cotyledons. (c) Decapitated at ca. 5 mm below cotyledons. (d) Intact control. (a)-(c) Right plants are controls.

<sup>1)</sup> The effect of removing the cotyledons differs of course according to the age of the seedlings, because their removal affects the nutrition as well as the growth substance.



may indirectly be caused by the inhibiting effect of hetero-auxin supplied in high concentration on the production of normal growth substance in the plant. But it seems to be less probable than the former.

#### IV. DISCUSSION

From the above experiments, the present writer has been led to the view that the growth inhibition and swelling of the *Helianthus* hypocotyl are caused by the excess of growth substance in the organ affected, although the detailed experiment as to the relation between the growth phenomena and the amount of growth substance has not yet been carried out.<sup>1)</sup> ZIMMERMAN and WILCOXON (1935) have reported that in seedlings of sweet pea and Windsor bean treated along one side with various growth substances in lanoline not only is the negative bending in the stem produced but there is also the abnormal enlargement of the stem tip and the elongation is retarded. They found also that, in artichoke and tobacco plants, the rate of growth of stems treated with growth substances in lanoline from the tip back to the base indicated first acceleration and then retardation of elongation. FISCHNICH (1935) has also reported retardation of stem-growth by hetero-auxin paste. LE FANU (1936) considered the growth inhibition in these cases to be the result of supplying a growth substance from a position morphologically basal to the region of elongation. She found that the growth of young internodes of *Pisum sativum* is inhibited by hetero-auxin lanoline pasted on the stem (*not decapitated!*) in a position morphologically below them, although their growth is accelerated by the same paste when applied to them from above (*after decapitation!*). She attributed the two opposing effects of hetero-auxin paste to the position where the paste was applied. But she has failed seemingly to notice the fact that the decapitation may play an important rôle in her experiments. LE FANU's results should more reasonably be explained by the amount of growth substance in the organ to be affected.

As already mentioned, the theory of CZAJA (1935) also cannot explain the results reported in the present work. It has been often reported that growth substances cause the growth in thickness in various organs of various plants (LAIBACH 1933 a, 1934 [—, MAI & MÜLLER], 1935, 1935 [— & FISCHNICH]; MAI 1934; MÜLLER 1935; SNOW 1935 [— & LE FANU], 1935; CZAJA 1935; SÖDING 1936). But such growth in thickness

<sup>1)</sup>[Addition during proof-reading]: Further experiments being under way suggest that the weak hetero-auxin paste applied to the cut surface of cotyledons, after removal of the upper halves, may accelerate the elongation of hypocotyl. (Duration of the exp. one day)

can hardly be considered to be caused by two streams of growth substance, flowing in different directions. The recent work of JOST and REISS (1936) also shows that the CZAJA's theory is not correct. They have found the following facts: Hetero-auxin paste when applied to the cut surface of the decapitated hypocotyl causes a swelling of the hypocotyl. But the reaction is different according to the species of the plant. In *Phaseolus vulgaris* a marked swelling occurs, while in *Lupinus* and *Helianthus* only a little swelling is caused. In the present work, however, the swelling was marked also in *Helianthus* when a seedling, decapitated just under the cotyledons, was used.

The works of FLIRY (1932), CZAJA (1935 a) and BRECHT (1936) show that the growth of the decapitated hypocotyl of *Helianthus* is accelerated by a growth substance applied to its cut surface. JOST and REISS (1936) show that a growth substance causes only a little growth acceleration in the decapitated hypocotyl of *Helianthus* but no effect in *Phaseolus vulgaris*. They suppose that the applied growth substance has no definite effect upon the growth of the decapitated hypocotyl, because the cells of these plants have sufficient growth substance or continue to produce it. They do not, however, maintain that in all the reports, attributing growth inhibition to a strong solution and acceleration to a weak solution, the conclusions arrived at are incorrect. They state, "Wir halten es für durchaus möglich, dass solche Wirkungen sich mit anderen Methoden, als wir sie verwandten, nachweisen lassen." Indeed, this problem has been cleared up to some extent by the results reported in the present work. But a quantitative study as to the growth substance in the hypocotyl is necessary to reach a definite conclusion.<sup>1)</sup>

## V. SUMMARY

1. The elongation of the *Helianthus* hypocotyl is inhibited by the hetero-auxin paste applied to the cut surface of cotyledons whose upper halves have been removed. At the same time, a marked swelling appears

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<sup>1)</sup>After the present paper was written, the writer received a paper from Dr. SNOW (1936). He reports that, when pea seedlings have a ring of hetero-auxin paste (1 in 1000) put round the stem close below one of the growing internodes, the elongation of this internode is at first accelerated (for less than 24 hours) and then strongly and increasingly retarded. He considers that the preliminary acceleration is presumably due to an upward movement of the hetero-auxin itself. With regard to the subsequent retardation, however, he seems to accept Miss LE FANU's view that the retardation may be an indirect effect of the growth substance.

on the hypocotyl. It starts abruptly several mm below the cotyledons.

2. The growth inhibition caused by the hetero-auxin paste occurs in the upper portion of the hypocotyl, while a slight acceleration is caused in its middle or lower portion.

3. When the hetero-auxin paste is applied to the cut surface of one of the cotyledons the upper halves of which have been removed, the hypocotyl shows negative curvatures in the middle or in the lower portion. Swelling occurs mainly on the side to which the paste has been applied, and the total elongation of the treated plant is inhibited. Similar results are found when the hetero-auxin paste is applied to one side of the hypocotyl of the intact seedling.

4. The inhibition of elongation in the upper portion of the hypocotyl is also caused when the hetero-auxin paste is applied to the cut surface of the hypocotyl, decapitated at about 1 mm below the cotyledons. On the other hand, if the decapitation is carried out at about 5 mm or more below the cotyledons, no inhibition, but rather an acceleration in elongation occurs. The swelling of the hypocotyl is remarkable only in the former case.

5. From the above facts, it is concluded that the inhibition of elongation and the swelling in the hypocotyl are probably caused by an excess amount of growth substance in the zone affected.

6. Root elongation is also inhibited by the hetero-auxin paste applied to the cut surface of the cotyledons.

The writer is greatly indebted to Prof. Dr. Y. YAMAGUTI for his kind direction in the course of the present investigation.

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# ON THE DISTRIBUTION OF THE ORIENTAL TERMITE, *COPTOTERMES FORMOSANUS* SHIRAKI, IN JAPAN

By

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(With Plate XII and two text-figures)

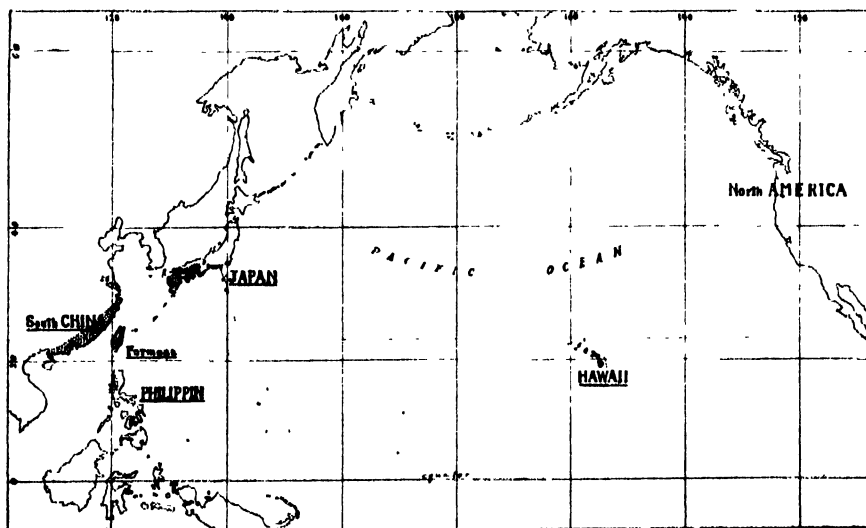
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## INTRODUCTION

There are many reports regarding the distribution of the Oriental termite, *Coptotermes formosanus* SHIRAKI, in Japan. Among these, two reports written by M. ŌSHIMA (1913) and M. YANO (1913) are noteworthy, while the remaining ones seem to be rather fragmentary.

In this paper, I shall deal with the same subject basing my conclusions on the results obtained by my own research and referring to those given in previous papers.

*Coptotermes formosanus*, the subject of this investigation, is a species



Text-fig. 1. A map showing the distribution of *Coptotermes formosanus* SHIRAKI in the world.

of termite, also called the Oriental termite or House termite, as it is widely distributed in the Orient, and is very destructive to house building.

It has been hitherto known that the species is especially sub-tropical in distribution, but extends even into the warmer area of the temperate zone, being found on the Pacific coast of southern China, of Formosa and of Hawaii, in the sub-tropical regions, and also in the southern parts of Japan, which belong to the temperate zone (Text-fig. 1).

The species, *Coptotermes formosanus*, was first described by SHIRAKI in 1909, the specimen having been obtained in Formosa. Afterwards, however, this termite was designated by various names by investigators being identified in a variety of ways as mentioned in the following list:—

<i>Coptotermes formosanus</i> .....	M. ÔSIHMA	(1912)
" .....	NITOBÉ	(1911)
" .....	HOLMGREN	(1913)
" .....	HÔZAWA	(1915)
<i>Termes raffrayi</i> .....	MATSUMURA	(1910)
<i>Termes (Coptotermes) formosanus</i> ....	M. ÔSHIMA	(1909)
<i>Termes (Coptotermes) gestroi</i> .....	M. ÔSHIMA	(1910)
<i>Coptotermes gestroi</i> .....	U. NAWA	(1910)
" .....	M. ÔSHIMA	(1911)
<i>Termes gestroi</i> .....	M. ÔSHIMA	(1911)
<i>Coptotermes</i> sp. ....	M. YANO	(1910)
<i>Coptotermes formosae</i> .....	HOLMGREN	(1911)
" .....	M. YANO	(1911)
" .....	U. NAWA	(1912)

Concerning the synonymy of the terms used for this species readers are advised to refer to HÔZAWA's papers (1912, p. 297; 1915, p. 92).

At present it retains the specific name first given by SHIRAKI, and is placed in the systematic position here given below.

Order .....	Isoptera
Family .....	Rhinotermitidae
Genus .....	<i>Coptotermes</i>
Species .....	<i>Coptotermes formosanus</i> SHIRAKI

Before proceeding further, I should like to express my sincere thanks to Professor Dr. SANJI HÔZAWA for the kind instruction and encouragement given me in the course of my investigation. I wish also to express my gratitude to Professor Dr. HIROSHI ÔHSHIMA of the Zoological Institute of the Kiushiu Imperial University, and to Professor Dr. TEIZO EZAKI of the

Entomological Institute of the same University for their valuable suggestions to me.

#### THE COLLECTION OF SPECIMENS

With the purpose of ascertaining the facts and conditions of the distribution of the Oriental termite in Japan, I have made four tours of investigation in the region where this species of termite may be found.

In the first tour, which I made in May of 1935, I visited Kumamoto, Kagoshima, and Miyazaki Prefectures in Kiushiu.

In the second and third, which were made in November of 1935 and in January of 1936, respectively, I visited Shizuoka Prefecture, Izu Province, and the island of Izu-Ōshima.

In the fourth, which was made in June of 1936, I travelled along the Pacific coast of Wakayama and Mie Prefectures.

In each of the tours above-mentioned, I was able to obtain many specimens of the Oriental termite by examining groups of wooden structures such as temples, shrines, and houses, and the dead wood of living trees. Specimens are obtainable rather easily in the plantations of pinetrees growing along the sea-shore.

#### DISTRIBUTION OF *COPTOTERMES FORMOSANUS* SHIRAKI IN JAPAN

In what follows, the distribution of *Coptotermes formosanus* SHIRAKI in Japan is discussed the arguments being based on the facts obtained either by myself or by previous investigators. The localities, where this kind of termite was found, are marked on the map (Pl. XII).

1. Kiushiu. In Kiushiu this species is widely distributed not only in the parts of the prefecture lying along the sea-coast but also nearly all over the region. However, it must be mentioned that the species has not been found until now in the country along the northern sea-coast of Miyazaki Prefecture.

2. Shikoku. As regards the distribution of this species of termite, Shikoku shows a type almost similar to that of Kiushiu. But I much regret that I am not able to say definitely whether this termite inhabits the region lying along the western and southern sea-coast of Shikoku, as I have not been able to go there yet.

3. Honshiu. As regards the distribution of this species in Honshiu, it seems likely that it is not so wide as in the two regions of Kiushiu and Shikoku this being the effect mainly of the lower temperature and partly of other factors.



a) Chugoku. The species seems to occur only in the warmer tracts of country lying along the Inland Sea in this region. The termite was collected at Yoshikawa, Ogôri, Mitaziri, Yanai, Iwakuni, Tokuyama, etc. in Yamaguchi Prefecture; at Itsukushima, Itozaki, Mito, etc. in Hiroshima Prefecture, and in the vicinity of Kasaoka in Okayama Prefecture.

But it is open to question whether Shimonoseki and its vicinity in Yamaguchi Prefecture is one of the habitats of *Coptotermes formosanus* or not.

b) Kinki. In Hyogo Prefecture, *Coptotermes formosanus* is found in the region lying between Akashi and Wadamisaki, but is rather rarely found there, as compared with Chugoku.

In Ôsaka Prefecture, it inhabits the sea-coast, extending southwards to Hamadera.

In Wakayama Prefecture, this termite inhabits the area lying all along the sea-coast, and I have collected specimens even at Kinomoto, which is situated in the southern part of Mie Prefecture. In these regions the climate is comparatively warm, being influenced by the Black current and the termite dwells in the same good conditions as are found in Kiushiu and Shikoku.

c) Chubu. In this region the species is distributed mainly in the region along the coast of the Pacific Ocean. In Aichi Prefecture it was reported as found only in a part of the Chita and Atsumi Peninsulas.

In Shizuoka Prefecture, the species is found along the sea-coast of Suruga Bay in the region extending from Lake Hamana to the Omaezaki, and also in that extending from Miho-Matsubara to Sodeshi.

It was reported by OKADA (1913) that Sodeshi is the northernmost limit of the distribution of *Coptotermes formosanus* SHIRAKI in this country. Thus in Honshiu the species is not found north of Sodeshi in Shizuoka Prefecture.

As regards the Izu-Shichito Islands, this species was reported as found in Hachijojima by HÔZAWA (1912, 1915). I was personally informed by Mr. YUASA of the Imperial Agricultural Experiment Station in Tokyo that he has obtained specimens of this termite from Miyakejima.

Judging by the facts above-mentioned, it may be safely concluded that the distribution of *Coptotermes formosanus* SHIRAKI in this country is restricted, on the whole, to the warmer regions lying along the coast of the Pacific Ocean, and also that the northernmost limit of the species is the vicinity of Sodeshi in Shizuoka Prefecture, in latitude 35°03' N., while the easternmost limit is Hachijojima, at longitude 139°50' E.

THE LIMITING FACTORS OF THE DISTRIBUTION OF  
*COPTOTERMES FORMOSANUS* SHIRAKI

It seems that there exist a number of factors, which limit the distribution of *Coptotermes formosanus* SHIRAKI. Among them, the climatic factor, especially the temperature, seems undoubtedly to be the most important its effect being to limit the distribution of this species of termite.

1) Temperature as a Climatic Factor.

The climate of this country is relatively mild throughout the year, and it is also certain that *Coptotermes formosanus* will tend to survive and multiply even in the temperate zone, where the winter temperature is not low enough to kill it. It may therefore be reasonably concluded that the winter temperature is the most important factor limiting the distribution of this species of termite. This conclusion was also reached by YANO (1913), and by KOFFOLD (1934), in their reports written on the species.

Referring to the climatic data prepared by the Central Meteorological Observatory of Tokyo during the period extending from 1910 to 1929, we find that in every place which this species of termite inhabits the average annual temperature is higher than 15°C. and at the same time, that the average temperature of January, the coldest month in winter, is higher than 4°C.

In Shizuoka Prefecture we found this species of termite at Miho, which is situated within the northern limit of its distribution, while we do not find it at Numazu which is outside the same limit.

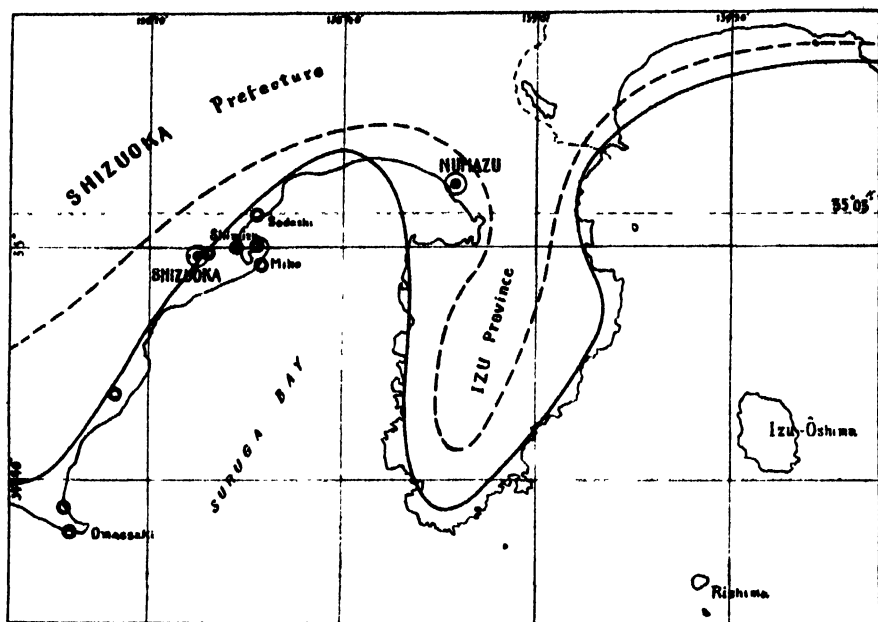
Both places lie quite close to each other, and have an average annual temperature higher than 15°C. and that of January being higher than 4°C., but yet they show a marked difference regarding the occurrence of *Coptotermes formosanus*.

The reason which causes the above peculiarity in the distribution, seems to be the difference in the minimum daily temperature, i. e. at Miho the monthly average minimum daily temperature for January is higher than 0°C., while at Numazu the minimum is lower than 0°C. (Text-fig. 2).

Judging by the facts above-mentioned, the existence of this species of termite in the northern parts of the temperate zone of Japan seems to depend on the daily alternation of temperature in the winter in addition to the annual changes of temperature.

I have tried to plot such places as to have a monthly average minimum

daily temperature in January higher than  $0^{\circ}\text{C}$ . and I discovered that the isothermal line prepared in such a way entirely coincides with the line showing the actual distribution of this species, as is clearly shown on Plate XII.



Text-fig. 2. A map showing the distribution of *Coptotermes formosanus* SHIRAKI in Shizuoka Prefecture. The heavy line is an isothermal line connecting the place which have a monthly average minimum day temperature for January of  $0^{\circ}\text{C}$ .; the heavy broken line shows an isothermal line connecting the places which have a monthly average temperature for January of  $4^{\circ}\text{C}$ .; the round marks indicate the habitats of this species of termite.

Thus we are inclined to conclude that in this country *Coptotermes formosanus* SHIRAKI inhabits the warmer parts, where the mean temperature of the coldest month, i. e. of January, is found to be higher than  $4^{\circ}\text{C}$ . and at the same time, the monthly average of the minimum day temperature of January is higher than  $0^{\circ}\text{C}$ .

## 2) Other Factors limiting the Distribution of *Coptotermes formosanus* SHIRAKI.

Though I have adopted the temperature as the main limiting factor in the distribution of this termite, but another climatic factor, such as humidity, should also be considered as having an important effect on the

life of this termite which lives underground and makes its nest below the surface of the earth.

The amount of moisture contained in the food to be taken by this species is usually directly affected by the moisture content of the soil, as the food is usually laid on the ground or underneath it. It is also a well established fact that when the termite swarms it needs an atmosphere almost saturated with water vapour. An experiment which was made by IWASAKI (1911) at Ishigakijima has shown that, when this kind of termite swarms, it needs an atmosphere containing at least 95 per cent. of moisture.

Other factors, on which the extension of the distribution area of *Coptotermes formosanus* depends, are the nature of the soil, the land features of the environment, and certain artificial means.

But the factors just mentioned are not so important as the temperature factor in their action in limiting the distribution of this species of termite.

#### SUMMARY

1) As the result of my four tours of investigation in various parts of Japan and taking into consideration the results obtained by previous authors, I have been able to demarcate the limits of the distribution of *Coptotermes formosanus* SHIRAKI in Japan.

2) The occurrence of *Coptotermes formosanus* in this country is confined by the temperature limit, to region which have a monthly average temperature for the coldest month, namely for January, higher than 4°C. and at the same time, the monthly average minimum day temperature for the same month higher than 0°C.

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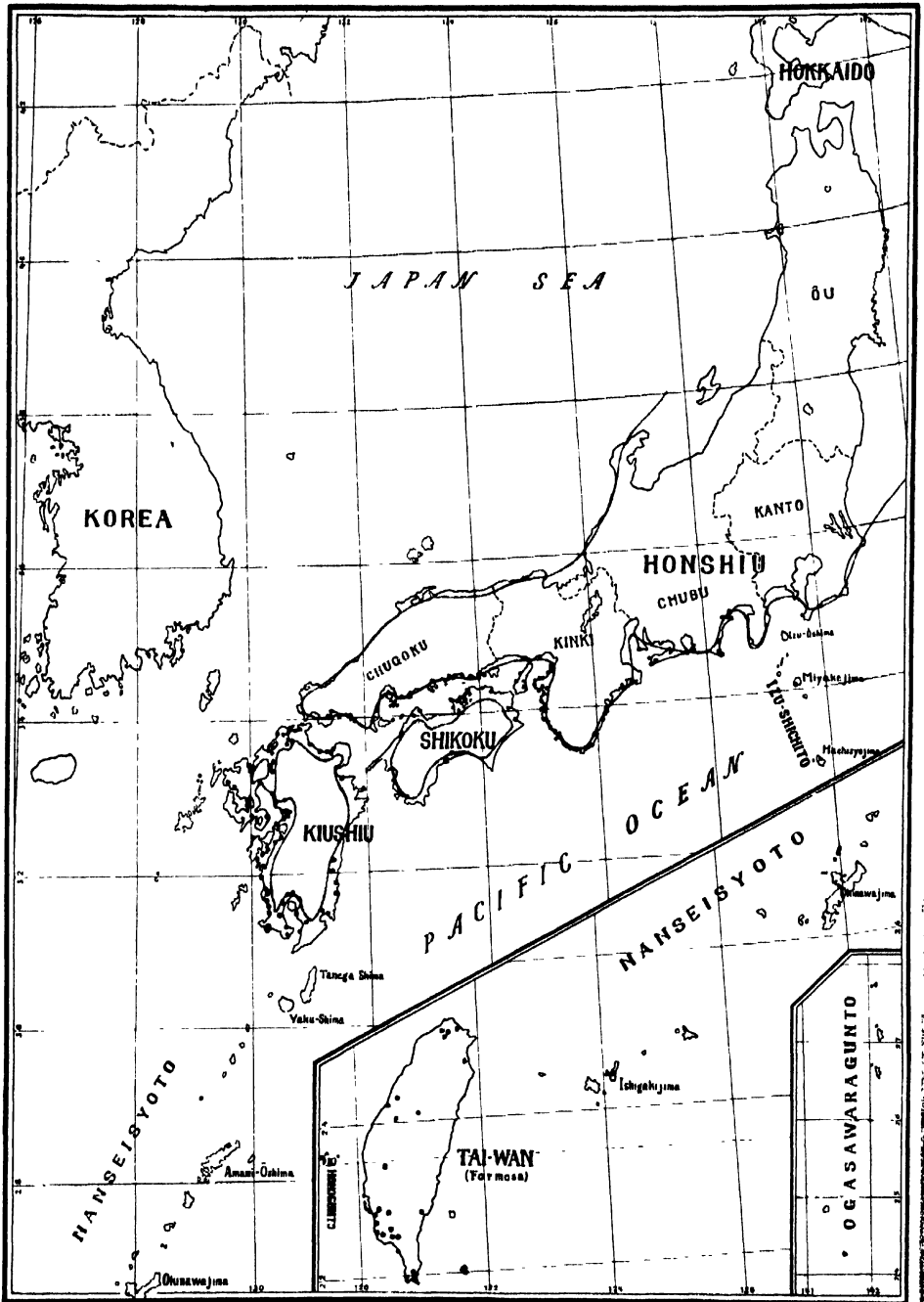
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## EXPLANATION OF PLATE XII

A map showing the distribution of *Coptotermes formosanus* SHIRAKI in Japan. The red spots indicate the localities where this termite is found; the blue line is an isothermal line connecting the places which have the same monthly average minimum day temperature for January in degrees 0°C.



Y. ABE del.

Y. ABE: Distribution of Oriental Termites.





# ON THE BREEDING HABIT OF THE EARTHWORMS WITHOUT MALE PORES

## I. ISOLATING EXPERIMENT IN *PHERETIMA HILGENDORFI* (MICHAELSEN)

By

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(Keiō Second Higher Common School)

(With one figure)

(Received March 15, 1937)

### 1. Introduction.

Since BEDDARD ('92) was struck with astonishment of finding the earthworm without male pores, *Perichaeta rokugo* (= *Pheretima hilgendorfi* (MICH.)<sup>1)</sup>), we know at present as many as ten species<sup>2)</sup> of such worms, all of which belong to the genus *Pheretima*, and most of them are found in Japan. It is a very interesting question how these worms can vigorously continue breeding. But, unfortunately our knowledge on this problem is meager.

YAMAGUCHI ('31) studied in detail the variability in occurrence and position of the male pores in *Ph. hilgendorfi*, and suggested that the breeding habit in this species may be cleared by the histological studies and feeding experiments. By the work of ÔISHI ('30), the reproductive processes of the earthworm of the genus *Pheretima* was made clear. But, it is hard to believe that such process also occurs in the worms without male pores. GATES ('32 and '33) as a result of his studies of the genitalia in the *Ph. anomala*-group, finds that the PICKFORD-STEPHENSON thesis ('29 and '29) is not applicable at least to this group. In certain respects this

<sup>1)</sup> *Ph. hilgendorfi* is widely distributed in both Japan and Korea. In Hokkaidō, it may extend to north of Sapporo. In Honshū, Shikoku, and Kyūshū, it as well as *Ph. communissima* may be said to be a common earthworm. In Korea, it is distributed in all except the far northern regions; in the east-northern region it is restricted in distribution to near Kankō, Kankyō-nan-dō, and in the west-northern region near Chinnampo, Heian-nan-dō. In the south of these districts, it is common in either towns, harbours or villages, but is rather rare or not found if remote from the formers. Considering from the distribution, its occurrence in Korea is probably a matter of human transference from Japan.

<sup>2)</sup> *Ph. hilgendorfi*, *Ph. agrestis*, *Ph. vittata* (Japan and Korea), *Ph. divergens*, *Ph. irregularis*, *Ph. parvula*, *Ph. schisopora*, *Ph. gunoshimensis* (Japan), *Ph. speiseri*-A-form (New Caledonia), and *Ph. anomala insolita* (India and Burma).

thesis may be said to be in common with HATAI's saying ('31, p. 29), "Even in the worms without male pores the deposition of the cocoons can be observed as in the normal species. But, it is not clear whether the eggs within those cocoons are fertilized or not, and whether the spermathecae of those worms are containing the spermatozoa in copulation with those with male pores". CERNOSVITOV ('27) reported a unique possibility of the self-fertilization in the earthworm, on individuals of *Tubifex tubifex* which lack the spermathecae.

In my recent paper ('36), I reported that the hatchability in *Ph. hilgendorfi* is as high as 84.4%. I was unable to find the copulation of any kind in the same species in the laboratory condition in my last two years' observation. So, I undertook to detect indirectly its breeding habit in several ways. And, as it appears to me that a key to this interesting problem has been obtained, I intend here to report the results as a preliminary.

Before going further I wish to express my hearty thanks to Dr. SHINKISHI HATAI of the Tôhoku Imperial University for his continual guidance to my study; and it is a pleasure to record here a debt of gratitude to Profs. HARUJIRÔ KOBAYASHI and TAMEZÔ MORI, of the Keijô Imperial University for their kindness and encouragement given me throughout this work.

## 2. Growth Rate of *Ph. hilgendorfi* in Keijô.

To know the life-history of *Ph. hilgendorfi*, the growth rate was examined. Five larvae hatched in the laboratory on March 24th, '36; these were continually fed and both their length and weight were measured week by week. At the same time, a same number of worms were collected at random in the field and also measured. The results are shown in Table 1 and Fig. 1.

In field experiment, in less than half of the worms collected in the 13th week (June 23rd), the clitellar glandularity was found to begin to appear; and the clitellum was completed in the 19th or 20th week (4th or 11th, August). The term of the completion of the clitellum means that the setae, intersegmental furrows, and dorsal pores on it are obliterated. (But, the dorsal pores on it are not infrequently obliterate even in the apparently completely matured worms.) Of course, some exceptions were found; for instance in some it was completed much later, in the 23rd week. If the completion of the clitellum is parallel in development

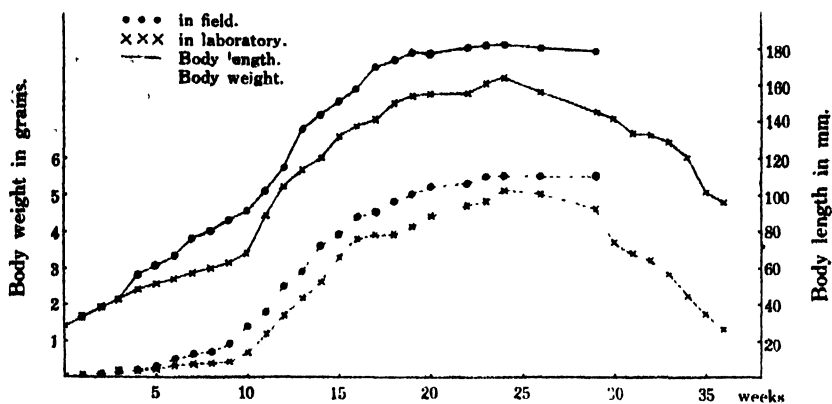


Fig. 1. Showing the growth rate.

TABLE 1.

to the maturity of the internal genital organs in normal conditions, it may be said that the present species reaches the full-grown stage after the 19th week or after the beginning of August in Keijō. Even after their attainment of the full-grown stage, both length and weight increase but very gradually during several weeks. Following this period, both length and weight begin to decrease very gradually, perhaps by the deposition of the cocoons. In 1936, no worms were found in the field in the 30th week; probably due in part to the heavy rain-fall during this week, thus, their death might be accelerated to some extent. (In

Weeks	Field		Laboratory	
	L (mm)	W (gr)	L (mm)	W (gr)
0			28	0.029
1	33	0.051	34	0.051
2	38	0.098	38	0.099
3	42	0.138	43	0.116
4	56	0.206	48	0.176
5	61	0.302	51	0.228
6	66	0.483	54	0.283
7	76	0.570	57	0.339
8	80	0.691	60	0.365
9	86	0.882	63	0.383
10	91	1.411	68	0.652
11	102	1.808	89	1.172
12	115	2.529	105	1.729
13	136	2.922	114	2.204
14	144	3.634	120	2.601
15	151	3.925	132	3.301
16	158	4.371	138	3.814
17	170	4.522	141	3.865
18	175	4.766	150	3.883
19	178	4.992	154	4.128
20	177	5.197	155	4.444
21				
22	180	5.321	155	4.740
23	181	5.476	160	4.810
24	182	5.484	164	5.073
25				
26	180	5.465	156	4.972
27				
28				
29	178	5.460	145	4.595
30	not found		141	3.689
31			133	3.410
32			132	3.225
33			128	2.825
34			120	2.240
35			101	1.686
36*			96	1.273
37			all died	

\*...in laboratory, four worms died.

1935, no worms were found on 8th, November.)

The length, weight and the increment of the worms in the laboratory are considerably smaller than those in the field. This may be due mainly to the difference of nourishment of the earth in which they live. In laboratory conditions, the clitellar glandularity began to appear a little earlier (about a week) than in the field, as in *Ph. sp.* studied by TAKAHASHI ('33) in Taihoku. The clitellum of four worms was completed in the 19th week, but in the remaining one much later in the 23rd week. The age and period of both maximum length and weight are nearly equal to those of the worms in field. After this period, both measurements decrease gradually until the 29th week, and then rather rapidly decrease until the time of the dissolving of their own bodies or of their death. Out of five worms, four died in the 36th week, and the other one in the next week (8th, Dec.). Before dying, the dissolving of the tail end of the body occurs first, and then it waves anteriorly to the region just behind the clitellum. The worms at this condition are rather motionless and insensitive unless some stimuli were applied to the preclitellar region. Before dissolving their body, the body wall becomes somewhat transparent compared with the normal ones. Then, the postclitellar region becomes rather flattened, and succeeding the anal portion gradually decreases in breadth (in flattened condition) — its appearance somewhat resembles the upper part of a flat whisky-bottle. Next, such process waves anteriorwards, as described above. Finally, the same process takes place also on the preclitellar region. The entirely destroyed corpses were always found in some depth of the earth.

The worms fed in the laboratory for the study of growth rate all lack the male pores.

In the field, at the 13th week was found a single worm having the male pore (a single pore) but without clitellar glandularity. The male pore is only represented as an indistinct whitish spot, being only slightly elevated from the general surface of the body. Presumably, it may not serve as a functional male pore, even among the matured worms having male pores, if the copulation occurs as in the normal species. On finding this worm, I immediately prepared the isolating experiment.

### 3. Ratio of the Individuals with and without Male Pores.

#### (a) In large collection :

In Keijô, I collected 622 worms (also including those shown in Table

3) within the last three years. Out of these, only 48 possessed male pores. Comparing this result with YAMAGUCHI's the following was found :

TABLE 2.

No. of worms	Without MP.	With MP.	Locality
1005	888	117(12%)	Sapporo
622	574	48( 8%)	Keijō

From the above result, it may be said that in a large collection the worms with male pores are only about 10% of the total number.

(b) In small area :

Out of 128 worms which were collected at random within the last three years from about twenty-eight localities of the Korean peninsula except Keijō, only seven were found to possess the male pores. If the PICKFORD-STEPHENSON thesis is applicable to the present species, both forms with and without male pores must be always found in a small area, for the worms would never wander about except under some unfavourable circumstances, and, according to OISHI ('30), in most worms even in the time of copulation their tail portion is retained within their burrows. In such meaning the ratio of both forms in a unit area was examined in several spots in Keijō.

TABLE 3.

No. of spots	No. of worms	Without MP.	With MP.	Area (Sq. M.)
1	6	6	0	2
2	9	9	0	2
3	13	13	0	3
4	37	37	0	3
5	12	11	1	2
6	22	21	1	3
7	66	64	2	3
8	17	15	2	1
9	31	29	2	1
10	33	30	3	3
11	61	57	4	3
12	78	71	7	3

From the result of this simple ecological study, the PICKFORD-STEPHENSON thesis appears to be not applicable to the present species. This idea introduced me to try the isolating experiment.

#### 4. Method and Materials of the Isolating Experiment.

##### (a) Brief description of the materials.

Seventy worms for the present experiment and several for the future study were collected on June 23rd, 1936, from about three square metres (No. 12 in Table 3), and the former number includes a worm with male pores which was collected from a separate but near spot.

In general, between both forms with and without male pores, no marked differences were found in both external and internal characteristics except the male pores and posterior male organs. On the variability in occurrence and position of both male pores and posterior male organs, YAMAGUCHI ('31) fully studied Sapporo-specimens. The result obtained from the Korean specimens is entirely included within his data.

(1) Male pores and posterior male organs. (i) In worms without male pores: no external openings of the sperm-ducts were found on any segment even in the sections, as in YAMAGUCHI's examination. Sperm-ducts on each side meet mostly in XIII and extend on parietes as far back as various segments of XVI-XXX (usually behind XVIII and mostly in XVIII-XXIV), and there terminate blindly with a simple bulbular swelling which is placed on parietes or very seldom only a trifle sunken into the parietes. (ii) In worms with male pores: Pores are mostly unpaired. One worm has two pores on one side on XVII and XVIII. Each pore is represented as an indistinct whitish spot, very slightly elevated from the general surface of the body; and it is quite different in appearance from those of the matured ones, in which it is situated on a markedly protuberant conical porophore with much elevated and apically wrinkled epidermis. The structure of the posterior male organs is much smaller than those of the matured ones, and is similar to those of the normal species of the genus; gland lobular, occupying about 3-5 segments; duct muscular, stout, moderately long, forming an O- or U-shaped loop; sperm-duct connected with the ental end of the prostatic duct. (2) Anterior male organs. Testis sacs in X and XI, much smaller than those of the matured ones, about  $1/3$ - $1/5$  of the latter; testes slightly smaller than the latter. Seminal vesicles in XI and XII, a little smaller than, or nearly equal to, those of the matured ones, but always flattened in the former and a little more voluminous in the latter. (Those of the latter are usually small proportional to the body size, as stated by BEDDARD ('92).) (3) Female pore and ovaries. In most worms a single female pore is scarcely visible midventrally on the setal line of XIV, and is represented as faintly

luminous and lip-like elevations with a slit-like opening. Ovaries in XIII, a little smaller than those of the matured ones. (4) Spermathecal pores are rather well-developed, and are easily visible as orangish spots in the intersegmental furrows of 6/7 and 7/8. Spermathecae are much smaller than those of the matured ones; the ampulla is not so wrinkled and not so semitransparent as in the latter, but is rather smooth and somewhat voluminous, containing whitish materials. In the serial sections of six spermathecae taken at random from six worms collected together with those used in the present experiment, no male cells at any stages were found within both ampulla and diverticulum. In all cases examined, in ampulla fine granules are compactly packed, and in diverticulum a very little large granules are found. (5) Clitellum. In less than half of the worms the clitellar glandularity begins to appear. (6) Genital papillae are rather well-marked in all worms.

(b) Method of isolating experiment.

As shown in Table 4, five series were planned. I-series: a single worm without male pores was put into each of five pots, A, B, C, D, and E. II-series: five worms without male pores were put into each of three pots, G, H, and I. III-series: four worms without male pores and

TABLE 4.

I-series.					
Pot No.	A	B	C	D	E
No. of worms	1	1	1	1	1
II-series.					
Pot No.	G		H	I	
No. of worms	5		5	5	
III-series.					
Pot No.	M		N	O	
No. of worms	4 & 1'		4 & 1'	4 & 1'	
IV-series.					
Pot. No.	P			Q	
No. of worms	15			15	
V-series.					
Pot No.				R	
No. of worms				5'	

1, 5, 4, 15....without male pores.

1', 5'.....with male pores.



a single one with male pore (or pores) were put into each of three pots, M, N, and O. IV-series: fifteen worms without male pores were put into each of two pots, P and Q. V-series: five worms with male pores (or pores) were put into a single pot, R.

Each pot used in I-series involves about three litres of earth; that in II-V-series five litres. The earth used in the experiment was collected from the place where the cocoons were found in spring, and was completely dried in the sun, since 26th, April, 1936. Through the experiment, the suitable volume of water was poured into every pot day by day, to support the constant moisture of the earth. Sometimes decayed leaves were put into every pot for manure of the earth. Except these two manipulations, the pots were never effected by any marked stimuli until the examination of them was made in winter. But, as they were set on the floor of the laboratory, the vibration by walkings must, of course, be taken into consideration.

## 5. Results of the Isolating Experiment.

### (a) Copulation was not observed.

Copulation of the worms in each series was not observed in both day and night conditions. In the period, from the end of September to several days before the worms die, the majority of the worms frequently creep with the greater part of the body but retaining a short tail portion within their burrows, or some entirely creep out from their burrows and wander about on the surface of the earth, even in the day-light condition. Frequently some of these worms are closely attached to one another. But, this attachment is never considered as the copulation of any kind from their behavior: in their attachment no rules are found, their heads are in most cases parallel in direction, and no noticeable movements of the genital regions are recognized.

### (b) Oviposition.

I failed to observe the real state of the oviposition. But, it may be said that the time of oviposition is the period from the middle of September to the beginning of October. The worm in B-pot died as early as in the 27th week (Sept. 29th); it is the first record of death of the worms in this experiment, but twenty-one cocoons were found in its pot. And the period above mentioned appears to agree with the results of observation in the field that six apparently fresh cocoons were found at first in this autumn on Sept. 20th, and that no worms were found on Oct. 20th,

as already described in the section of growth rate.

Most of the larvae within the cocoons obtained in this experiment were fully-developed, and besides, several ones which had been hatched were found when the examination of the pots was made, as shown in Table 5. The larvae within these cocoons are nearly equal in development to those found in December in the field. But, from oviposition to hatching, about twenty-eight weeks are required in the field, while in laboratory a little more than half of this duration. Such delay of hatching in the former might be caused mainly by the cold weather.

(c) Cocoons in each series.

With no exception, in all the pots the cocoons containing the normal larvae were found. The details of the results are summarized in Table 5.

TABLE 5.

Pot No.	No. of cocoons	No. of cocoons containing larvae	% of cocoons containing larvae	Mean value of Dia. (mm) W. (mgr) of cocoons		Supposed date of death of the last worm	No. of larvae which had hatched	Date of examination
A	51	42	82.4	3.7	27	1/XII	3	22/XII
B	21	18	85.7	3.5	24	29/IX	1	10/XII
C	25	17	68.0	3.7	27	6/X	0	4/XII
D	18	1	5.6	3.6	23	4/XII	0	20/XII
E	13	8	61.5	3.7	26	6/X	0	21/XII
G	61	54	88.5	3.7	27	24/XI	9	24/XII
H	71	66	93.0	3.7	26	10/XI	1	11/XII
I	50	44	88.0	3.6	24	24/XI	0	19/XII
M	60	50	83.3	3.6	25	10/XI	0	1/XII
N	61	50	82.0	3.5	23	10/XI	5	23/XII
O	68	58	85.3	3.6	25	10/XI	4	18/XII
P	38	35	92.1	3.5	23	20/X	0	20/XI
Q	136	120	88.2	3.5	22	27/X	0	27/XI
R	37	36	97.3	3.6	22	1/XII	1	15/XII
	710	599	84.4	3.6*	24*			

Dia. .... long plus short axes/2.

\* .... from 657 cocoons.

The most remarkable fact obtained from this experiment is that the cocoons containing the normal larvae were found in each of I-series. The cocoon number in this series varied from 13-51, and of which those containing the larvae 1-42. Out of eighteen cocoons found in D-pot, only one contained the larva. Causes of such results are not clear at present; (the worm in this pot died latest, on 4th, Dec.). This case is much lower than any of the others in percentage of the number of cocoons containing the larvae. Among the latters no marked difference was found concerning

the same fact. The average number of cocoons deposited by all seventy worms is 10, and that of each series varied from 6-26. The mean value of diameter of the cocoons in each series is nearly equal to each other, but that of weight is slightly variable. The mean percentage of the total number of cocoons containing the larvae is 84.4%. This value is fortuitously quite the same as the hatchability obtained in my spring experiment ('36). The mean value of the diameter of the cocoons is a little larger than that of the spring experiment; 3.6 mm in the former and 3.3 mm in the latter. The larger diameter in the former is perhaps due to the absence of much smaller ones as in the latter.

In the pot in which the worms were fed for the study of growth rate, two larvae which had been hatched and five cocoons which contained only the albuminous fluid were found at the examination of Dec. 17th. Such small number of cocoons deposited by five worms was probably caused by the frequent manipulation of the earth in measuring their length and weight.

Although several suggestive results other than above mentioned were obtained from this experiment, it will be unnecessary here to refer to them.

## 6. Discussion.

Considering from the disposition of the genitalia in the genus *Pheretima*, it must be a quite unusual phenomenon that the worms without male pores can vigorously continue breeding. And also, this may be a more unusual case than that in the worms without spermathecae (and spermathecal pores) but with male pores, such as some *Pheretimas*, *Lumbricid* and *Tubificid* worms. But, we must remember the study of ČERNOSVITOV ('27) on the possibility of self-fertilization in individuals of *Tubifex tubifex* which lack the spermathecae.

How can the earthworms without male pores continue breeding? At first this problem attracted BEDDARD's attention ('92) in examination of *Ph. hilgendorfi*. In spite of the fact that this interesting theme was thrown out before us, no one has ever made much effort to solve it. After the publication of both MICHAELSEN's ('92) and BEDDARD's ('92) papers on the description of this species, several Japanese species which similarly lack the male pores were reported again by GOTÔ and HATAI ('98 and '99), and by HATAI ('30). MICHAELSEN ('13) reported from New Caledonia *Ph. speiseri* which includes the two types A and B, the former without male pores but with well-developed spermathecae and the latter with male

pores but with reduced spermathecae. In 1929, PICKFORD reported from New Hebrides the C-form of the same species which is normally hermaphrodite. In this year, STEPHENSON subdivided *Ph. anomala* into three types, *insolita*, *typica*, and *centralis*. Then, the PICKFORD-STEPHENSON thesis attracted the attention of the zoologists. But, by the intensive works of GATES ('32 and '33), it was pointed out that this thesis is not applicable at least to *Ph. anomala*-group. ÔISHI ('30) reported a very interesting fact on the reproductive processes in the genus *Pheretima*. Considering from the disposition of the genitalia, such process may be universal in this genus. But, it is hard to believe that the similar process also occurs in the worms without either male or spermathecal pores. YAMAGUCHI ('31) fully studied *Ph. hilgendorfi*, the variability in occurrence and position of the male pores, and suggested that the breeding habit in this species may be cleared by histological studies and feeding experiments. HATAI ('31) reported that the deposition of the cocoons can be observed even in the worms without male pores, as in the normal species, and that it is not clear whether the eggs within those cocoons are fertilized or not. In my recent paper ('36), I reported that in *Ph. hilgendorfi* the cocoon number in a square meter is 327 and the hatchability is as high as 84.4%.

*Ph. hilgendorfi* is widely distributed in both Japan and Korea, and its hatchability is 84.4% as described above. From this fact, I think the special method of the breeding habit must occur in this species. According to the studies of YAMAGUCHI and my own, in a large collection of *Ph. hilgendorfi* there are found the worms with male pores in about 10%. I think both forms with and without male pores must be always found in a small area if the PICKFORD-STEPHENSON thesis is applicable to the present species. Because, the worms would never entirely creep out from their burrows except under very unfavourable circumstances, and according to ÔISHI, most of the worms in copulation still retain their tail portion within their burrows. But, in a small area both forms with and without male pores are not always co-habitated. Thus, I thought that the copulation might not occur between both forms with and without male pores, (the former being "functional male" and the latter being "functional female"). To prove this assumption, the following experiments were made: whether both isolated series of (1) a single worm without male pores and (2) several ones also without male pores, can deposit the cocoons containing the normal larvae. From these experiments, excellent positive results were obtained.

Although it is improbable that the copulation usually occurs among

the immatured worms of the present species, some attentions were paid to the content of the spermathecae of the worms which were collected together with those used in the isolating experiment.

Finally, I think, by the following experiments the breeding habit of *Ph. hilgendorfi* will be made clear: (1) seasonal examination of the content of spermathecae, (2) migration of the male cells, and (3) karyological studies of both somatic and germ-cells.

## 7. Summary.

1. Some preliminary experiments on the breeding habit of the earthworms without male pores were made on *Pheretima hilgendorfi* (MICHAELSEN), in Keijô.

2. The worms hatch at the beginning of April, and reach the full-grown stage after the beginning of August, and die at the beginning of November or the end of October. Before they go to death, the dissolving of the body occurs first on the tail end, and such process waves anteriorwards.

3. In a large collection, the worms with male pores are found in about 10%. But, in a small area both forms with and without male pores are not always co-habitated.

4. Copulation of no kind was observed.

5. Oviposition occurs in the period from the middle of September to the beginning of October. From oviposition to hatching, it requires about twenty-eight weeks in the field, while in laboratory a little more than half of the duration.

6. Isolating experiment was made. Aclitellate worms were isolated. Five series were planned: I-series, a single worm without male pores; II-series, five worms without male pores; III-series, four worms without male pores and one with male pores; IV-series, fifteen worms without male pores; V-series, five worms with male pores.

(a) A single worm without male pores can deposit the cocoons containing the normal larvae. Cocoon number varies from 13-51; of which those containing the farvae 1-42 (5.6-85.7%).

(b) Independently of the presence or absence of the worms with male pores, they can deposit the cocoons containing the normal larvae. Cocoons containing the larvae vary from 82.0-97.3%.

(c) Total number of cocoons deposited by seventy worms is 710. Percentage of those cocoons containing the larvae is 84.4%. This value

is quite the same as the hatchability obtained in the spring experiment. Average number of cocoons in each series varies from 6-26.

(d) Average value of the diameter and weight measured on 657 cocoons are 3.6 mm and 24 mgr, (diameter — long plus short axes/2).

7. Worms without male pores which were fed in laboratory being isolated from the time of larval stage, also deposited the cocoons containing the normal larvae.

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